THIS ISSUE IS DEDICATED TO DR. ESMOND R. LONG

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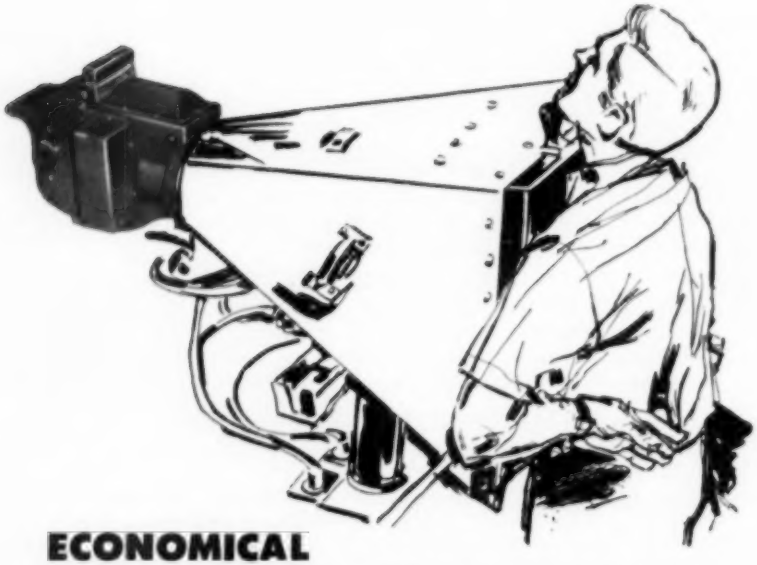

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The REVIEW wishes to dedicate this number to DR. ESMOND R. LONG, its former Editor-in-Chief and permanent Consulting Editor.

This number was originally chosen for the dedication because Dr. Long is retiring next month from his positions as Director of Medical Research of the American Trudeau Society and Director of the Henry Phipps Institute of the University of Pennsylvania.

The emphasis of a personal dedication necessarily rests on what a man *has* done. Hence, no matter how felicitously it may be phrased, a dedication carries with it the implication that not much more is to be expected from that man in the future.

In the present case, the subject has been quite unaware that this dedication was planned. Thus, in all innocence, he more or less confounded the dedicators by submitting for publication a report on some current laboratory investigations carried on by him and his associates (page 609). To confound the dedicators even further, their subject's studies have been conducted with certain of the most modern basic techniques, including the use of radioactive isotopes.

In short, Dr. Long, in his quiet and wholly innocent way, refuses to stay static and unproductive long enough to be "dedicated" for what he *has* done. This is perhaps as good a reason as the many others why the REVIEW wishes to dedicate this number, with the greatest affection and respect, to its former Editor-in-Chief.

March 28, 1955 THE EDITOR

THE AMERICAN REVIEW OF
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AND
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THE CARBON METABOLISM OF THE TUBERCLE BACILLUS

Studies with Isotopic Carbon[1,2]

ESMOND R. LONG, RUDOLPH J. ANDERSON, DAVID RITTENBERG, MANFRED L. KARNOVSKY, AND HOWARD J. HENDERSON

(Received for publication January 4, 1955)

INTRODUCTION

The investigation here described was designed to determine the manner in which the tubercle bacillus utilizes certain simple foodstuffs in the production of its own energy and the biosynthesis of its structural elements and characteristic products. It was hoped that some of the chemical pathways followed in the anabolic phase of growth and multiplication and the catabolic phase of metabolism could be identified. It was believed that establishment of fundamental knowledge on these phases of the metabolism of the tubercle bacillus would be valuable as a basis for further investigations on the mechanisms concerned in drug susceptibility and resistance and variations in the virulence of different strains of tubercle bacilli.

The project was organized to make use of tracer carbon in the form of carbon[13] and carbon[14] for the purposes named. Many investigators have used isotopic carbon in studying bacterial metabolism. Chiamori, Smith, Nishihara, and Winzler (1) have reported the utilization by the H37Rv strain of tubercle bacillus of acetate containing carbon[14] in the carboxyl position and glucose with all carbon atoms labeled. Lipids containing carbon[14] were found to be synthesized by the bacillus. They found the synthesis was not markedly affected by the presence of streptomycin.

In the experiments here described, a study was made of carbon dioxide production and synthesis of characteristic components of the bacillus from chemical units known to be of fundamental significance in the growth and metabolism of

[1] From the Henry Phipps Institute, University of Pennsylvania; Sterling Chemistry Laboratory, Yale University; College of Physicians and Surgeons, Columbia University; Biophysical Laboratory, Harvard University.

[2] Aided by grants in aid of research from the Office of Naval Research, the Atomic Energy Commission, and the National Tuberculosis Association.

the microorganism. For this purpose three compounds were used: acetic acid, glycerol, and carbon dioxide.

PLAN OF INVESTIGATION

As lipids comprise a major part of the tubercle bacillus and since acetate has repeatedly been shown to be the precursor of the "active acetate" involved in fatty acid biosynthesis, isotopic acetate, labeled in either the methyl or carboxyl group, was used in these studies as the first of the compounds of fundamental interest.

For this purpose carbon[13] was employed. Sodium acetate in the form of $CH_3C^{13}OONA$ and $C^{13}H_3COONa$, in separate experiments, was incorporated in simple, nonprotein media in a series of growth trials in which tubercle bacilli were cultivated on the surface of the medium.

The second of the two fundamentally important chemical compounds employed was glycerol. The growth-promoting properties of this substance for the human-type tubercle bacillus have long been recognized (2). It is common knowledge that glycerol influences the synthesis of the peculiar fatty substances in which the bacillus is rich, but there has been no precise understanding of the manner in which it does this. Studies in which a label is located at known positions in the substrate molecule should be ideal for the solution of this problem. With this objective, glycerol was labeled with carbon[14]. The latter was introduced for separate experiments in two positions in the molecule, viz., the alpha position ($C^{14}H_2OH \cdot CHOH \cdot CH_2OH$) and the beta position ($CH_2OH \cdot C^{14}HOH \cdot CH_2OH$).

The third compound studied was carbon dioxide. It is well known that carbon dioxide may be fixed by metabolizing systems, and it was considered of considerable interest to determine to what extent this occurs in the tubercle bacillus, into what compounds $C^{14}O_2$ is incorporated, and the position of incorporation of the fixed carbon in these compounds.

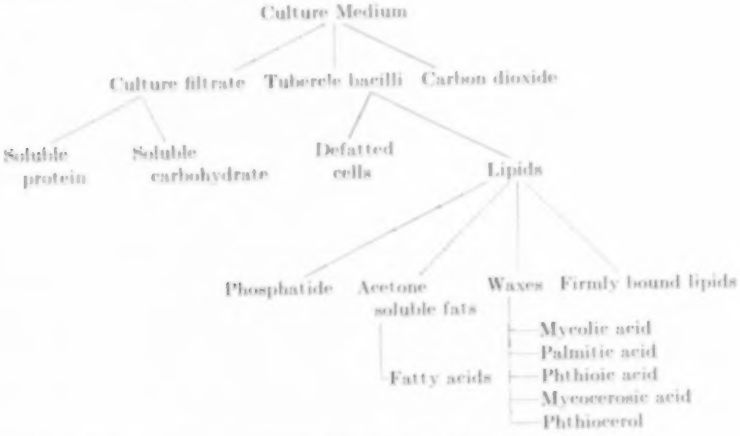

FIG. 1. Scheme of the fractionation of bacilli and products for analysis

In all of these experiments, tubercle bacilli were inoculated on the surface of Long's synthetic medium in which the substances named were incorporated, and after six weeks' growth the cultures were collected and analyzed. The experiments were carried out in closed systems. In the studies with C^{13} acetate and C^{13} glycerol, the carbon dioxide produced during bacillary growth was collected as sodium carbonate or barium carbonate.

At the end of each experiment the bacilli were filtered off and fractionated chemically. The soluble products of growth in the culture medium, viz., soluble protein and polysaccharide, were also collected and purified. A general scheme for the fractionation is shown in figure 1. Chemical and isotopic analyses were made to determine the presence and amount of isotopic carbon in the products of growth and metabolism, including the carbon dioxide produced.

The project was carried out as a cooperative enterprise in which the following participated in the manner indicated. The media were prepared with isotopic carbon constituents by H. J. Henderson and E. R. Long at the Henry Phipps Institute. These investigators grew the bacilli, collected the carbon dioxide, separated the bacilli from the culture filtrates and, with the cooperation of F. B. Seibert, isolated the soluble protein and carbohydrate.

C^{13} labeled acetate was obtained from a commercial source. The bacilli from the experiments with C^{13} labeled acetate were sent to R. J. Anderson at the Sterling Chemistry Laboratory, Yale University, for extraction and fractionation of the lipids. The fractionation was carried out by methods developed in the course of an extensive investigation of the chemistry of the tubercle bacillus with special reference to the lipid components (3). The lipids, extracted bacillary bodies, soluble protein and carbohydrate and carbonates from the carbon dioxide produced were analyzed by David Rittenberg and Laura Ponticorvo at the College of Physicians and Surgeons of Columbia University.

In the experiments with glycerol and carbon dioxide, the preliminary steps as outlined above were made by Henderson and Long at the Henry Phipps Institute and the bacilli, soluble protein and carbohydrate and carbonates were sent to M. L. Karnovsky at the Biophysical Laboratory of Harvard University, who, with his associates, analyzed all products for the presence and amount of carbon[13]. Dr. Karnovsky also prepared the C^{13} labeled glycerol and C^{13} labeled carbon dioxide used in the culture media (4).

RESULTS

Metabolism of C^{13}-labeled acetic acid: Experiments with separate labeling of the carboxyl and methyl carbon atoms of acetic acid showed that during growth carbon dioxide was produced in large quantity from both of these radicals. In this connection it should be noted that acetic acid does not appear to be used when it is supplied to the tubercle bacillus as the sole source of carbon (5). When incorporated in media with other sources of carbon, however, as in the experiments here reviewed, it appears to be metabolized readily. When analyses were made for other growth products than carbon dioxide in such experiments, carbon[13] was found widely distributed in the bodies of the bacilli, their lipids and structural elements, and in the soluble proteins and polysaccharides produced in their growth. Thus, both the methyl and carboxyl radicals of acetic acid enter into the major carbon cycles concerned in the metabolism of the tubercle bacillus.

In the utilization of acetic acid carbon for the production of its characteristic components, the bacillus appeared to concentrate it in the lipid to a greater extent than in its protein and carbohydrate elements. However, it was still a conspicuous part of the latter two fractions. The result conformed with the accepted view that acetic acid serves as a building block, particularly for the fats of the bacillus, and that other sources serve in greater degree for the proteins and carbohydrates.

TABLE 1

UTILIZATION OF THE CARBON OF ACETIC ACID BY THE TUBERCLE BACILLUS

Position of C^{13}	Atom Per Cent Excess C^{13}	
	$C^{13}OOH$	$C^{13}H_3$
No acetate used	30.7	23.2
Carbon dioxide produced	4.31	1.7
Soluble protein	2.56	3.48
Soluble carbohydrate	0.89	1.13
Fatty components of bacillus		
Phosphatide	5.26	2.64
Acetone soluble fat	3.25	2.34
Chloroform soluble wax	4.31	3.37
Firmly bound lipids	4.30	2.76
Mycolic acid	3.16	3.01
Palmitic acid	2.59	2.71
Phthioic acid	1.97	2.24
Mycocerosic acid	2.20	2.32
Phthiocerol	2.01	2.52

Fractions isolated for the purposes of this series of experiments are indicated in figure 1. In a typical pair of experiments, carbon[13], supplied respectively in the carboxyl and methyl radicals of sodium acetate, was found widely distributed in specific components of the bacillus, as indicated in table 1.

Metabolism of C^{13}-labeled glycerol: Experiments with separate labeling of the alpha and beta carbon atoms of glycerol showed that glycerol also serves as a source both for energy and synthesis of all of the major components and characteristic products of the bacillus. Regardless of its position in the glycerol molecule, the carbon[13] of glycerol was readily burned, appearing in large quantity in the expired carbon dioxide. Glycerol carbon was found also in all of the major fractions of the bacillus isolated after growth on labeled glycerol. In addition to a large amount of the original glycerol, carbon was found at the end of the experiment in compounds of low molecular weight, which are for the most part still unidentified. In certain of these experiments, the glycerol supplied (3 per cent by weight of the original culture medium) was completely used up.

At the end of the experiment, in one series, the distribution of the original glycerol carbon was as follows:

> 15 per cent appeared as carbon dioxide, 15 per cent entered the lipids and body structure of tubercle bacilli, 2 per cent appeared as soluble protein (tuberculin), 4 per cent as soluble polysaccharide, and approximately 50 per cent as compounds of low molecular weight (sugars, amino acids, and other metabolic products) in the culture medium.

The high percentage in the form of compounds of low molecular weight was believed to be of special significance and will be the subject of future investigation.

Metabolism of C^{14}-labeled carbon dioxide: In the experiment to study carbon dioxide fixation, it was found that carbon[14] from the $C^{14}O_2$ appeared to a considerable extent in the soluble protein (tuberculin) and to a lesser extent in the soluble polysaccharide. It was present also in the lipids and body structure of the organism. These findings are illustrated by the following data, based on two experiments in which growing tubercle bacilli were supplied with carbon dioxide labeled with carbon[14]. The specific activity of the carbon dioxide recovered from the flasks at the termination of the experiment, i.e., residual $C^{14}O_2$ originally introduced in the flask plus carbon dioxide produced by the bacillus from its substrate, is set arbitrarily at 100. On this basis the specific activities of the carbon of the dried bacilli in the two experiments were 2.5 and 7.5. Those of the soluble protein carbon were 5.0 and 20.0; and those of the soluble polysaccharide, 0.7 and 0.05. The differences reflect the stage of growth at which the carbon dioxide was introduced. During the course of the experiment, the specific activity of the $C^{14}O_2$ introduced in the flask was diluted twenty-five fold by carbon dioxide produced from the substrate by the organism.

DISCUSSION

The program has been successful in demonstrating the value of the methods used and indicating what steps should be taken to obtain precise information on the nature of the syntheses involved in formation of the constituents of the bacillus and the source of the principal metabolic products.

The results throw further light on a fact long appreciated, viz., the extraordinary capacity of the tubercle bacillus to build complex substances from very simple ones. The distribution of labeled carbon in all fractions studied confirms previous evidence indicating that the synthetic process for each of the characteristic compounds starts with very small chemical molecules, rather than large aggregates, and emphasizes the advisability of further studies on those enzymes of the bacillus concerned with the digestion and hydrolysis of the larger molecules present in the food supply of the bacillus in nature.

It was claimed long ago that some carbon dioxide is necessary for the growth of the tubercle bacillus. In the studies here reported, it was found that carbon dioxide fixation is important and, as might be expected from results with other organisms, the protein fraction proved most concerned in the fixation.

The investigation has laid useful groundwork for further advance in studying compounds of low molecular weight produced in the metabolism of the bacillus. These are present in large amounts in six-week cultures of the bacillus and may represent both degradation products of previously synthesized larger molecules in the bacillus and modified constituents of the medium still to be built into structural material. Work is in progress to determine the location of radioactive carbon in individual, selected, pure isolated sugars, amino acids, and fatty acids produced by hydrolysis of larger molecules. Data on compounds obtained from growth on different labeled substrates should provide useful information on the biosynthetic pathways available to the tubercle bacillus. Identification of some

of the compounds may furnish leads on nonutilizable analogues of products normally produced which might be of therapeutic interest.

The technical details of the experiments here reported and others to follow will be reported in subsequent papers.

SUMMARY

Isotopic carbon supplied to growing tubercle bacilli in separate experiments in the form of carbon[13] in the methyl and carboxyl groups of acetic acid, as carbon[14] in the alpha and beta positions of glycerol, and as carbon dioxide became widely distributed in the body of the tubercle bacillus and its soluble products in the course of bacillary growth.

A relatively high proportion of the labeled carbon of acetic acid and glycerol was ultimately found in the bacillary lipids. A significant proportion of the carbon[14] of labeled carbon dioxide was fixed by the bacillus, particularly in the soluble protein produced during growth.

Carbon dioxide containing labeled carbon was given off in conspicuous amounts when tubercle bacilli were grown in separate experiments on labeled acetic acid and glycerol, indicating extensive consumption of these substances in the course of metabolism.

SUMARIO

El Metabolismo Carbónico del Bacilo Tuberculoso Estudiado con Carbono Isotópico

El carbono isotópico suministrado a los bacilos tuberculosos en vías de desarrollo en distintos experimentos en forma de carbono[13] en los grupos metílico y carboxílico del ácido acético, en forma de carbono[14] en las posiciones alfa y beta del glicerol y en forma de bióxido de carbono, se distribuyó ampliamente en el cuerpo del bacilo tuberculoso y en sus productos solubles en el transcurso del desarrollo bacilar.

En los lípidos bacilares, se encontró por fin una proporción relativamente alta del carbono rotulado del ácido acético y del glicerol. Una proporción importante del carbono del bióxido de carbono rotulado fué fijada por el bacilo, en particular en la proteína soluble producida durante el desarrollo.

Se emitió bióxido de carbono que contenía carbono rotulado en cantidades notables cuando se cultivaron bacilos tuberculosos en distintos experimentos en ácido acético y glicerol rotulados, indicando esto extenso consumo de esas substancias en el transcurso del metabolismo.

RESUME

Etude sur le métabolisme du carbone du bacille tuberculeux à l'aide de carbone isotope

Le carbone isotope fourni aux bacilles tuberculeux pendant la phase de croissance, au cours d'expérimentations séparées, sous la forme de C^{13} dans le méthyle et le carboxyle de l'acide acétique et sous la forme de C^{14} dans la position alpha et bêta du glycérol et à l'état d'anhydride carbonique, a été rapidement dispersé dans le corps du bacille de Koch et dans ses produits solubles pendant la période de développement.

Une proportion relativement élevée du carbone marqué de l'acide acétique et du glycérol a été en dernier lieu découverte dans les lipides bacillaires. Une proportion importante du C^{14} de l'anhydride carbonique marqué était fixée par le bacille, en particulier dans la protéine soluble produite durant le développement.

L'anhydride carbonique renfermant le carbone marqué était éliminé en quantité re-

marquable quand, dans des expériences séparées, les bacilles tuberculeux étaient cultivés dans un milieu contenant soit l'acide acétique soit le glycérol marqué, ce qui indiquait une consommation extensive de ces substances au cours du métabolisme.

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PRIMARY SEROFIBRINOUS PLEURAL EFFUSION IN MILITARY PERSONNEL[1,2,3]

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INTRODUCTION

Primary serofibrinous pleural effusion, so-called "idiopathic pleurisy with effusion," has always been a malady not only of great clinical interest and importance but also of somewhat "uncertain" and, therefore, intriguing etiology and pathogenesis. Not to go back further than Koch's discovery (1882) of the tubercle bacillus, it is plainly evident that doctors for a long time have been struggling to determine whether acute pleurisy with effusion was or was not a benign affair, or was or was not due to infection with the tubercle bacillus.

The present paper is a report of 141 cases of primary serofibrinous pleural effusion in young adult white men with five or more years of follow-up observation. It indicates the tuberculous nature of this form of pleurisy and emphasizes the importance of adequate immediate treatment and long-continued observation to prevent relapse. Full appreciation of the clinical problem can be gained from a review of previous observations.

Historical Review

Frank Donaldson,[6] writing in Pepper's *A System of Practical Medicine by American Authors* (1885) (1), summarized current opinions and his own. Quoting Bartholow, he wrote: "The importance of pleuritis as a cause of phthisis is hardly sufficiently recognized in inducing tubercular deposit, and by adhesion limiting the movements of the organs, and thus inducing diseases." Again, he quoted Anstie to the effect that "it is now well established not merely that pleurisy often occurs in phthisical lung disease, but that pleurisy itself is capable of setting up true tuberculosis even in previously healthy persons." Donaldson added: "Modern authors thus consider that a productive field is offered for the bacillus tuberculosis."

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[2] This study was part of a project initiated in January, 1944, with Dr. James J. Waring as Responsible Investigator, Colonel George F. Aycock and Colonel Esmond R. Long as consultants, and Lt. Colonel William H. Roper as Director of the Section.

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Donaldson was on less uncertain ground when he wrote:

> Tubercular pleurisy may be acute or chronic. It may occur during the course of ordinary tubercular disease of the lung by extension of the disease from the lung to its serous covering, or it may proceed from tubercular deposit in the pleura independently of any previous disease of the lung.

The serious significance of bilateral pleural effusion was also clear to him:

> It rarely happens in primary acute pleurisy that both pleurae become involved. When such is the case, however, it is generally tubercular in its nature and necessarily a very grave if not a fatal complication. Walshe reports having seen four such cases of idiopathic bilateral pleurisy in persons physically healthy and perfectly free from constitutional taint of any kind. In all the pericardium was involved and in one the peritoneum. They were all fatal.

H. F. Vickery,[7] in a fine paper (1887), "Pulmonary Tuberculosis as a Sequel to Ordinary Pleurisy with Effusion" (2), put the matter very clearly in an opening sentence: "The object of this paper is to show the possibility of a pleurisy with serous effusion being, clinically, the first step towards consumption, or, in other words, that a man who has heretofore been apparently sound and falls ill with acute pleurisy, may be destined, finally, to succumb to pulmonary tuberculosis." He quoted Strumpell as saying: "In an etiological sense, we must declare the large part of the ordinary 'pleuritic effusions' which, clinically, seem to be primary, to be tubercular." Vickery added: "Indeed, some have claimed that every pleurisy is tubercular in its origin." He said in conclusion: "Coming now to the practical lesson, it is briefly this: In all cases of pleurisy with effusion, long after apparent recovery, the patient is in danger of pulmonary tuberculosis, and demands all possible hygienic precautions."

B. F. Westbrook (3), in his paper (1888) on "Pleurisy as a Predisposing Cause of Phthisis Pulmonalis," showed a remarkably clear appreciation of both the clinical problem and the underlying pathology of tuberculous pleurisy. He wrote:

> Pleurisy has been so frequently observed as an antecedent, either immediate or remote, of tubercular disease of the lungs, that it has not failed to attract the attention of clinicians from the earliest times, and there has been much discussion as to the true relationship which the two diseases have to each other.

Giving illustrative case reports, he then classified pleurisy antedating phthisis as follows:

1. Those in which a pleurisy with effusion, arising in a person apparently in good health, is immediately followed by evidences of pulmonary tuberculosis which usually runs a rapid course to its fatal termination. This is the phthisiogenic tubercular pleurisy *d'emblée* of Renaud.

[7] H. F. Vickery was one of the translators of Strumpell's *A Textbook of Medicine for Students and Practitioners*, 1886-1887. Adolf Strumpell (1853-1925) was Professor of Medicine at several German universities, finally at Leipzig. He described various new disease pictures, such as spondylitis deformans, acute hemorrhagic encephalitis, and so-called "Westphal-Strumpell's" disease.

> It is a form of the disease which is frequently observed both in hospital and in private practice, both of which I have seen many instances. . . . Under this head might also be included the cases reported by Knaevenagel and Jaccoud in which caseous foci are found in the lungs immediately subjacent to the inflamed pleura, from which foci Jaccoud maintains that auto-infection results.

2. This class includes those cases in which a serofibrinous pleurisy is followed by the slow development of chronic interstitial pneumonia—the fibroid phthisis of Sir Andrew Clark. The future development of distinct tuberculosis in such cases is to be looked for.

3. Cases in which all acute or subacute attacks of serofibrinous pleurisy, terminating in complete recovery, are followed after some months by the development of tuberculosis in one or both apices.

4. Cases in middle age or later in which a serofibrinous pleurisy has become chronic. While many live for many years without serious results, "it is nevertheless a fact that some of them finally develop tuberculosis, either in the lungs alone or in a general disseminated form."

5. Finally, those cases of empyema the appearance of which antedates the occurrence of tuberculosis.

Westbrook's conclusions are:

1. Serofibrinous pleurisy, simple in origin and terminating in complete recovery so far as local manifestations are concerned, may be followed after many months by phthisis pulmonalis.

2. In all probability the pleurisy in these cases acts as a predisposing cause of the tuberculosis. (Compare Fishberg's comment on "predisposing" on page 622.)

3. Primary serofibrinous pleurisy may result in fibroid phthisis with subsequent tuberculosis pulmonum.

4. The natural deduction from these facts is that no case of pleurisy should be neglected. In the words of Sir Andrew Clark: "When we have a patient with basic fibrinous pleurisy, let us hold him fast, restrict his freedom, and treat him carefully until every remnant of it is gone."

Close on the heels of these farsighted papers of Vickery and Westbrook came the 1889 presidential address (4) before the American Climatological Association of Vincent Y. Bowditch (1852–1929) on "Comparative Results of Ninety Cases of Pleurisy, with Special Reference to the Development of Phthisis Pulmonalis." This carefully documented paper represents an analysis of ninety cases of pleurisy which occurred from 1849 to 1879 in the practice of Henry I. Bowditch[5] (1808–1892), the father of Vincent Y. Bowditch. The writer's aims were to show how many regained their health and how many succumbed later to phthisis or "other probably tubercular disease." Since this paper is impressive in its follow-up ob-

[5] Henry Ingersoll Bowditch (1808–1892), Harvard Medical School graduate in 1832, pupil of P. C. A. Louis, chiefly remembered, according to Garrison, for his advocacy of paracentesis thoracis for pleural effusions (Am. J. M. Soc., 1852, *23*, 320), his translations of the works of Louis (1836), his book *The Young Stethoscopist or The Student's Aid to Auscultation* (1846), "probably the best and most detailed description of early pulmonary tuberculosis that had appeared in America or indeed in any country" up to this time, according to Lawrason Brown.

servations and is furthermore quoted by Osler in several editions of his *Principles and Practice of Medicine* (5), it is summarized here in some detail.

Bowditch began with some references to the opinions of other distinguished specialists of the day. Notable is the reference to Landouzy (1845–1917), of the Paris Faculty of Medicine, who held the "most extreme" view that nearly all cases of pleurisy are tuberculous and that this so-called "simple" pleurisy from "taking cold" is a very rare thing, but that the great majority are the expression of an incipient pulmonary tuberculosis which may appear at any subsequent time, even after an interval of many years. "In this opinion he is enthusiastically followed by his pupils, J. R. G. Joanney and Alois Mayor, who give Landouzy's views in two theses upon this subject."

In his address, Bowditch lumped together all cases of pleurisy, including "dry pleurisies, serofibrinous and empyemas," since he wished to "cast out" every case in which there was the "least evidence of co-existing pulmonary disease (for we all know that pleurisy is a frequent accompaniment of phthisical tubercle)." He divided the thirty-year period of observation from 1849–1879 into three decades.

In brief, in the first two decades (1849–1870), approximately two-thirds of those who died of phthisis succumbed in seven years or less, while one-third lived ten to eighteen years longer. Of the total of ninety patients with pleurisy, 31 or 34 per cent died of phthisis and one more was "phthisical."

Bowditch considered "most unwarrantable the assertion, that because pleurisy develops in any subject who, several years before, had had pleurisy, the two diseases in every case are necessarily dependent upon each other, the intervening period having been one of robust health." He ended his report on an optimistic note, "A large percentage of pleurisy patients recovered their health and never had any recurrence."

William Osler's thoughts about the relationship of tuberculosis to "idiopathic serofibrinous pleurisy" are presented in the first edition (1892) of his *Principles and Practice of Medicine* (5). He said:

> For generations physicians have considered cold the potent factor in inducing pleurisy. This may be true in many cases, but modern views of serous inflammation scarcely recognize cold as anything more than a predisposing agent, which permits the action of various micro-organisms.
>
> Of late years an attempt has been made, particularly by French writers, to show that the majority of acute pleurisies are tuberculous. In this connection the following facts must be admitted: (*1*) In a limited number of cases of pleurisy coming on abruptly in healthy persons, the disease has been shown—(*a*) by a post-mortem, in cases of accidental or sudden death, (*b*) by the subsequent history—to be tuberculous; (*2*) in a larger proportion of these cases which come on insidiously on persons who have been in failing health or who are delicate, the disease is tubercular from the outset; (*3*) the acute pleurisy, which occurs as a secondary, often a terminal, event in chronic affection, such as cirrhosis of the liver, Bright's disease, and cancer, is very frequently tubercular. I confess that the more carefully I have studied the question the larger the proportion appears to be of primary pleurisies of tuberculous origin. The subsequent history of cases of acute pleurisy forces us to conclude that in at least two thirds of the cases it is a curable affection. This may well be so according to our present ideas of local tuberculous disease.

In the fourth edition of his book (1901) Osler (5) said that he had "reviewed the post-mortem records of 101 successive patients who had died on his wards with pleurisy—fibrinous, serofibrinous, hemorrhagic or purulent." Only thirty-two were definitely phthisis. He quoted Hedges' figures of 40 per cent of one hundred and thirty patients with primary pleurisy with effusion as becoming tuberculous after seven years of observation. In brief, Osler said "Bacillus tuberculosis is present in a very large proportion of all cases of primary or so-called idiopathic pleurisy."

John B. Murphy (6), in his famous lecture on "Surgery of the Lung," delivered in Denver in June, 1898, before the American Medical Association, said German writers think "simple" pleurisy is tuberculous pleurisy, the true nature of which is not often recognized. Murphy himself was of the opinion that tuberculosis was the etiologic factor in pleurisy and represented the underlying cause of more than three-fourths of the cases.

Germain Sée (1818–1896) (7), a distinguished French Professor of the Faculty of Medicine of Paris, believed that three-fourths of all pleurisies were tuberculous, and quotes Fiedler, who said that only twenty-one patients recovered of one hundred and twelve with pleural effusion requiring aspiration. Of the other ninety-one patients, twenty-five died of phthisis at home or in the hospital and sixty-six recovered from the pleurisy but were found to be victims of "confirmed phthisis" or other tuberculous disease. Sée did not think that pleurisy *a frigore* or idiopathic pleurisy was due to the primary direct effect of cold but that chilling only favored its development by "permitting the real cause to act more rapidly or more efficaciously."

In 1902, Richard C. Cabot (8) read a paper before the Association of American Physicians on "The Prognosis of Pleurisy with Serous Effusion." This was an important study of one hundred and fifty-four patients with pleural effusion followed by letter and personal visit after their apparent recovery. He concluded this paper: "So far as my statistics go the cases may all be of tuberculous origin." At the present day, it is worth comment that thirteen, nearly 10 per cent of Cabot's one hundred and fifty-four cases, "contracted" tuberculosis five to sixteen years later. Cabot's use of the word "contract" is disturbing. Did he mean to imply that the subsequent appearance of tuberculosis was a new infection or the relapse of a disease the first manifestation of which was pleurisy with effusion, or was he in doubt?

In the discussion of Cabot's paper, M. L. Harris said:

> In England pleurisy is certainly regarded very seriously by the life insurance companies, and it's the rule now not to accept any applicant who has had pleurisy within a period of five years. It is also the custom to regard any case of pleurisy occurring in young subjects as of tuberculous origin, and that a large proportion of these subjects will within five years become claims upon the societies as the result of tuberculosis.

Very little comment on pleurisy with effusion can be found in Arnold C. Klebs *Tuberculosis, A Treatise by American Authors*, published in 1909. The words "pleurisy" and "effusion" do not appear as major items in the index. Ludwig

Hektoen (9) in the chapter on "Tubercle and Morbid Anatomy" wrote: "In the case of the pleura and the pericardium, general tuberculosis may arise either from the tuberculous pulmonary foci or from foci in the thoracic lymph nodes." Charles L. Minor (10), in his chapter on "Symptomatology of Pulmonary Tuberculosis" said:

> A pleural form of acute miliary tuberculosis is seen occasionally beginning either secondarily with effusion and high fever or gradually. Unlike simple pleurisy there are marked toxic symptoms and it is apt to end suddenly in meningitis. The fact that the dyspnea is out of proportion to the amount of the effusion may assist in the diagnosis.

Pleurisy with effusion and its relation to tuberculosis received much more consideration in S. G. Bonney's *Pulmonary Tuberculosis and Its Complications*, second edition, published in 1910 (11). Bonney wrote that:

> It has been known for many years that a large majority of the so called idiopathic pleurisies are tuberculous in character. While many pleurisies, either with or without effusion, end in apparent recoveries, some are followed after the lapse of months or years by the development of pulmonary tuberculosis. It is not very uncommon to observe patients whose cough, expectoration, fever, and physical signs immediately follow the acute pleural invasion or so shortly thereafter as to justify their classification as cases of acute onset characterized by an initial pleurisy.

Of 2,070 recorded cases of pulmonary tuberculosis, Bonney found one hundred and twenty-six presenting the history of an idiopathic pleurisy as the first symptom referable to the disease. In sixty-seven patients (3.2 per cent of 2,070), the pleural effusion preceded other pulmonary signs and symptoms. In forty-two, the pulmonary symptoms developed after the lapse of two years or more following the pleurisy; in eight, from one and one-half to two years; in four, from one to one and one-half years; in nine, from six to twelve months; and in four, slightly less than six months after the pleurisy. One hundred and twenty-six other patients referred the onset of the pulmonary condition to an *accompanying* pleurisy. "It is safe to assume," said Bonney, "that an exceedingly large proportion of idiopathic serous effusions are actually tubercular in character" (p. 446).

* * *

Brief extracts from the works of more recent writers may now be given to show that the most competent and experienced phthisiologists were just about ready to discard the word "idiopathic" as applied to acute serofibrinous pleurisy. In 1917 Landis (12), in that valuable book on diseases of the chest (Norris and Landis) said: "While a primary pleurisy can be produced by exposure to cold, it is now known, as I shall show later, that the majority of the so-called idiopathic cases are in reality tuberculous in nature" (page 527). In the 1924 edition, he wrote: "So long as there is doubt as to which are really primary and which are secondary to a latent tuberculosis, it is unwise to assure the patient that the trouble is trivial in nature." From the records of the Phipps Institute, Norris and Landis reported that 23.8 per cent of 5,895 patients with pulmonary tuberculosis gave an antecedent history of pleurisy.

F. T. Lord (13), in his *Diseases of the Bronchi, Lungs and Pleura*, in 1925, found in 1,185 cases of serofibrinous pleurisy that the pleuritis was unassociated with positive findings elsewhere in 750 persons (63.4 per cent). The pleural disease was combined with suspected or definite pulmonary or other tuberculosis in 160 patients (13.5 per cent). The lung was involved in 149 persons, with tubercle bacilli in the sputum of 47. Tuberculous peritonitis was present in 9 and tuberculosis elsewhere in 2 of the group.

Albert Calmette (14), in his *Tubercle Bacillus Infection and Tuberculosis in Man and Animals*, in 1923 said: "It (acute serofibrinous pleurisy) appears chiefly in individuals who have no evident past history of tuberculosis." (An implication of the primary or post-primary nature of acute serofibrinous pleurisy)

Lawrason Brown and Heise (15), in 1931, said: "Pleurisy with effusion should always make us suspect the presence of tuberculosis and demand a thorough examination of the chest."

M. Fishberg (16) in his book *Pulmonary Tuberculosis* (1932) from his experience said:

> A pleural effusion is very often the first indication of phthisis. . . . Strictly speaking, pleurisy cannot be considered predisposing to pulmonary tuberculosis, because it is in itself a manifestation of tuberculous disease. . . . I have observed a number of cases of pleurisy with effusion which began with hemoptysis. In fact, in several cases the disease was ushered in with profuse pulmonary hemorrhage. All those turned out to be tuberculous. . . . It has been my practice to consider all pleurisies accompanied by hemoptysis as tuberculous. . . . Are all pleurisies tuberculous? The most important prognostic problem in these cases is whether the patient, after recovering from pleurisy, will develop pulmonary tuberculosis, and if so, what effect will the pleural lesion have on the immediate and ultimate outlook for recovery. Experience has taught that a large proportion of patients with pleurisy ultimately develop phthisis.

Pagel, Simmonds, and MacDonald (1953) say that a considerable proportion of persons with idiopathic pleural effusion later have tuberculous disease of the lungs or other organs (17). They quote Gsell (1930) to the effect that the presence of tuberculous changes in approximately 80 per cent of all cases of pleurisy and the demonstration of tubercle bacilli in the pleural fluid strongly suggest a tuberculous etiology in most cases of "simple" pleural effusion (page 346). Clinical evidence supports pathologic evidence that the typical "idiopathic" pleural effusion is tuberculous. Pagel and associates summarize by stating that the frequency of pleural effusion in the antecedent history of patients with pulmonary tuberculosis, the evidence of the tuberculin reaction, the similarity of these effusions to those occurring in confirmed tuberculosis, the high proportion of such fluids which contain tubercle bacilli, and the subsequent history of patients with pleural effusion all unite to confirm the opinion that so-called primary serofibrinous pleural effusion is of tuberculous origin.

According to these writers, pleurisy with effusion may develop under the following conditions:

1. During the formation of a primary focus. Primary foci which develop as they so commonly do in the immediate subpleural area of the lungs are drained not only by the

lymphatic flow toward the hilum but also by lymphatic channels toward the pleura, pathways so beautifully demonstrated by William Snow Miller.

2. As a late primary manifestation. Here the effusion appears in 30 to 50 per cent of instances within six months after infection.

3. Pleurisy with effusion, which may be unilateral, found in approximately 20 to 40 per cent of cases of disseminated tuberculosis.

> It is either an independent tuberculous manifestation in the pleural membrane (nodular, caseous, or exudative), or a collateral (non specific) hypersensitive reaction to an intrapulmonary spread which may be negligible in size and extent. Evidence can be adduced for the role of allergic hypersensitivity in the development of pleurisy in disseminated tuberculosis.

Pagel, Simmonds, and MacDonald believed that idiopathic pleural effusion must be treated as due to active tuberculosis in the absence of clear evidence for another cause. It is likely to be followed by pulmonary tuberculosis in a fair proportion of cases.

Finally, Capps and Capps (18), in *Oxford Medicine*, say: "In all probability the majority of these pleurisies are not truly primary but represent the localized flaring up of an old inflammatory process in the neighboring tissue of lung or glands."

* * *

From this historical review, certain conclusions may be drawn. Although the association between pleurisy with effusion and phthisis (tuberculosis) was noted by many good clinicians, the tendency in some quarters seemed to be to cling to uncertainty, as evidenced by the retention of the words "idiopathic" and "predisposing"; faith here and there in the major, if not etiologic, influence of cold (pleurisy *a frigore*); and inclusion of empyemas in case reports. Some of the empyemas may have been tuberculous or pyopneumothorax, but doubtless many more were of nontuberculous origin. It was clear to many students, however, that tuberculosis was the fundamental cause of acute serofibrinous pleurisy with effusion. Moreover, the late, even remote, appearance of phthisis (tuberculosis) after acute pleurisy with effusion was not overlooked. It should be recalled that the roentgenogram was not discovered until 1895 and that, in 1910, most of the sanatoriums were just beginning to appreciate the value of the roentgenogram in chest diseases. Moreover, it was probably not until the nineteen twenties that great help was being given the chest physician by this method of examination. Hence, all praise must be given the earlier physicians quoted in this review for their astute observations.

OBSERVATIONS

Composition of group: The present report includes initial (January, 1944, to early 1945) and five-year follow-up observations (until late 1950) on 141 military personnel in whom acute primary serofibrinous pleurisy with effusion without evidence of pre-existing tuberculous disease occurred during service in the Army. Manifest tuberculosis did not develop in the lung or elsewhere in any one of the 141 persons within eight months after onset of the initial pleurisy. During the

original illness none had been treated with chemotherapy, for these drugs were not yet available. The fundamental purpose of the present analysis is to throw some light on the cause and the subsequent history of so-called primary pleurisy with effusion.

The material of the study consisted of 141 previously healthy white male officers and soldiers who were admitted to various U. S. Army hospitals in 1944 and early in 1945 with pleural effusion of unknown cause. The starting point of observation is taken as the onset of the pleuritis. In each instance, special study by the Research Section was initiated soon after the onset and without advance knowledge regarding the patient's cutaneous reaction to tuberculin.

Earlier pre-effusion roentgenograms were reviewed in all cases. In a few instances, when the earlier roentgenograms suggested that active pulmonary disease existed prior to or at the time of the effusion, these patients were excluded from the survey. Likewise, excluded in certain calculations were a few (13 patients) who developed pulmonary parenchymal or extrapulmonary disease while still under treatment for the effusion. On follow-up, in no instance did a pulmonary lesion develop later in that area which originally was obscured by the fluid.

All of the 141 patients reacted positively to intracutaneous purified protein derivative (PPD); 126 patients (90 per cent) reacted to first-strength PPD and 15 (10 per cent) to the second-strength PPD. Twelve patients also reacted to coccidioidin and 38 to histoplasmin, but the tuberculous etiology of the pleuritis in all of these persons was proved by other means.

In summary, the selection of patients was based on unexplained serofibrinous pleural effusion persisting for more than four to six weeks in young white male adults in the Army, without advance knowledge of tuberculin reactions and without concentration on those already tuberculosis suspect.

Period of follow-up observation: Six of the 141 persons died within three years after onset of the disease: 5 from pulmonary tuberculosis, but all with extrathoracic tuberculosis as well; and one man died from other causes. He had already developed active pulmonary tuberculosis before he died of carcinoma of the larynx. Frequent follow-up observations (at least four per person per year) were completed on each of the remaining 135 persons for five or more years after onset of the original pleural effusion. The 141 persons were studied for a total of 804.3 years (9,652 months) after the onset of the disease, an average of 5.7 years for each person. A total of 5,583 chest roentgenograms were reviewed, averaging 39.6 for each person, and the minimum number for any person was 31.

Age at onset: Of the 141 persons with serofibrinous pleural effusion, 92 (65.2 per cent) later developed some form of active tuberculosis (table 1). The probability of subsequent appearance of disease was higher in the younger age group: 75.3 per cent among the 73 who were under twenty-five years of age when they first developed effusion, and 54.4 per cent among the 68 who were twenty-five years or older.

Bacteriology of fluids: Of the 42 persons whose original pleural fluids were not examined adequately by culture or animal inoculation, 71.4 per cent later developed active tuberculosis; 60.4 per cent of 48 with sterile fluids by culture

TABLE 1

PATIENTS WITH PRIMARY SEROFIBRINOUS PLEURAL EFFUSION WHO SUBSEQUENTLY RELAPSED WITH TUBERCULOSIS, CLASSIFIED BY AGE AT ONSET

Age at Onset	Patients with Primary Serofibrinous Pleural Effusion		
	Total	Those Who Subsequently Relapsed with Tuberculosis	
		Number	Per Cent of Total
All ages	141	92	65.2
18 to 24 years	73	55	75.3
25 to 45 years	68	37	54.4

TABLE 2

PATIENTS WITH PRIMARY SEROFIBRINOUS PLEURAL EFFUSION WHO SUBSEQUENTLY RELAPSED WITH TUBERCULOSIS, CLASSIFIED BY BACTERIOLOGY* OF FLUID

Bacteriology* of Fluid	Patients with Primary Serofibrinous Pleural Effusion		
	Total	Those Who Subsequently Relapsed with Tuberculosis	
		Number	Per Cent of Total
Total	141	92	65.2
Positive	51	33	64.7
Negative	48	29	60.4
Test not made	42	30	71.4

* Culture and/or guinea pig inoculation.

and/or guinea pig test later developed active tuberculosis; 64.7 per cent of 51 whose original fluids were positive for tubercle bacilli subsequently developed tuberculosis (table 2). In one patient, four cultures and two guinea pig inoculations of concentrated fluid were negative for tubercle bacilli. On later decortication, the pleura was found studded with tubercles, some of which contained virulent tubercle bacilli. These observations indicate that routine bacteriologic studies of pleural effusion may be of doubtful dependability for either etiologic diagnosis or prognosis.

Hematologic findings: Leukocytosis and elevation of erythrocyte sedimentation rate were found irregularly in some patients. However, these findings did not show significant correlation with the frequency of the subsequent appearance of tuberculosis.

Volume of fluid: The estimated maximum volume of fluid as revealed by the roentgenogram was of no value in differential diagnosis or in prognosis. Forty-nine of these patients had *small* volumes, i.e., extension up to the sixth rib anteriorly, and 67.3 per cent of the group developed tuberculosis; 72 patients had *moderate* volumes, i.e., extension up to the fourth rib, with 62.5 per cent developing tuberculosis; 20 had *large* volumes extending above the fourth rib, with a 70 per cent incidence of subsequent tuberculosis. In general, the patients with

TABLE 3

PATIENTS WITH PRIMARY SEROFIBRINOUS PLEURAL EFFUSION WHO SUBSEQUENTLY RELAPSED WITH TUBERCULOSIS, CLASSIFIED BY MAXIMUM VOLUME OF FLUID AS ESTIMATED BY ROENTGENOGRAPHY

Maximum Volume of Fluid	Patients with Primary Serofibrinous Pleural Effusion		
	Total	Those Who Subsequently Relapsed with Tuberculosis	
		Number	Per Cent of Total
Total	141	92	65.2
Small: to sixth rib	49	33	67.3
Medium: to fourth rib	72	45	62.5
Large: above fourth rib	20	14	70.0

smaller volumes were treated for the same periods as those with *larger* effusions (table 3).

Persistence of fluid: Effusions resorbed more rapidly in some patients than in others. Again, this finding was of no assistance with regard to etiology or prognosis. In this study, the usual time required from onset of the pleurisy for complete resorption ranged from approximately two months for the small effusions to four months for the larger ones.

Treatment. Influence of bed rest: In correlating the duration of treatment with subsequent developments, 13 of the 141 cases were excluded in order to confine observations only to 128 patients who had apparently recovered fully from their initial attack of pleurisy before release from treatment. The 13 patients referred to in this paragraph all developed pulmonary parenchymal tuberculosis or extrathoracic tuberculosis, or both, during their original hospitalization. In no instance, however, did the disease first make its appearance in less than nine months following the onset of effusion. The pulmonary lesion developed on the same side as the pleural effusion in 3 patients, on the opposite side in 5 (one with extrathoracic tuberculosis also), and on both sides with coincidental extrathoracic tuberculosis in 2 patients. Finally, in the remaining 3, recurrence consisted in extrathoracic involvement without manifest pulmonary lesions. Two of the six extrathoracic relapses consisted in tuberculosis of the knee joint.

Except for the above 13 patients, the duration of original Army hospitalization depended upon contemporaneous military directives. The directives specified the disposition of patient personnel of various categories and, hence, did not allow unrestricted medical decision. Of the remaining 128 patients, none was "ill" beyond the first few months after onset and none evidenced relapse with active tuberculosis during the initial period of treatment. In no instance was the duration of the original hospitalization influenced by initial volume, rapidity of resorption, or bacteriologic studies of the pleural fluid, or by the degree of toxicity in the early illness. Triple correlation of the bacteriologic findings with the duration of treatment and with the ultimate outcome, i.e., relapse with active tuberculosis, did not alter the significant relationship between the duration of treatment and fre-

quency of subsequent relapse, as reported immediately below. Excluding 4 patients who died during their original hospitalization, all of the 141 persons resumed military duty or returned to full-time civilian work within a month or two after release from the Army hospital.

Sixty-six of the remaining 128 patients underwent varying degrees of bed rest for less than six months, with a mean of four and eight-tenths months; 60 or 90.9 per cent of these later developed tuberculosis within five years after onset. Sixty-two patients rested for six or more months, with a mean of ten and four-tenths months; 19 patients (30.6 per cent) developed tuberculosis within five years; 43 (69.4 per cent) remained well for at least five years thereafter (table 4).

Residual pleuritis: The term "post-treatment roentgenogram" refers to roentgenograms taken after apparent recovery from the original illness with effusion.

The immediate post-treatment roentgenograms of all of the 141 patients were free of pulmonary parenchymal shadows. In 10 instances, the post-treatment chest roentgenogram was "negative" or normal; 70 per cent of these persons later developed active tuberculosis. Eighty-three showed slight residual pleuritis, usually indicated by slight blurring of the costophrenic angle and possibly with

TABLE 4

PATIENTS WITH PRIMARY SEROFIBRINOUS PLEURAL EFFUSION WHO DID NOT RELAPSE DURING ORIGINAL HOSPITALIZATION BUT WHO SUBSEQUENTLY RELAPSED WITH TUBERCULOSIS, CLASSIFIED BY MONTHS OF REST

Months of Rest	Patients with Primary Serofibrinous Pleural Effusion Who Did Not Relapse During Original Hospitalization		
	Total	Those Who Subsequently Relapsed with Tuberculosis	
		Number	Per Cent of Total
Total	128	79	61.7
Less than 6 months (mean = 4.8 months)	66	60	90.9
6 months or more (mean = 10.4 months)	62	19	30.6

TABLE 5

PATIENTS WITH PRIMARY SEROFIBRINOUS PLEURAL EFFUSION WHO SUBSEQUENTLY RELAPSED WITH TUBERCULOSIS, CLASSIFIED BY EXTENT OF RESIDUAL PLEURITIS

Extent of Residual Pleuritis	Patients with Primary Serofibrinous Pleural Effusion		
	Total	Those Who Subsequently Relapsed with Tuberculosis	
		Number	Per Cent of Total
Total	141	92	65.2
Heavy	9	5	55.6
Moderate	39	27	69.2
Slight	83	53	63.9
None	10	7	70.0

a thickened pleural cap over the apex of the lung; 63.9 per cent of these developed active tuberculosis. Thirty-nine had moderate residual pleuritis and 9 heavy, with respective tuberculosis incidence rates of 69.2 per cent and 55.6 per cent (table 5).

Nature of relapse: Reactivations developed later in 92 of the 141 persons. The relapses represented virtually all known forms of the disease, in almost all parts of the body. This further showed that tuberculosis is a generalized disease in spite of its predilection to appear more often in a few selected tissues or organs (table 6).

The relapse was proved bacteriologically or pathologically as *tuberculous* in 87 of the 92 patients concerned. In the remaining 5 patients, adequate bacteriologic and pathologic studies were not done or were negative; but the clinical, roentgenographic, and other findings justified a presumptive diagnosis of tuberculosis.

Ninety-two persons later had reactivation of tuberculous disease. Forty-nine (53.2 per cent) had pulmonary parenchymal recurrences only. Fourteen (15.2 per cent) had recurrence with both pleural effusion and parenchymal disease; 10 had recurrent pleural effusion only; 8 had recurrence with pulmonary parenchy-

TABLE 6

PATIENTS WHO DEVELOPED TUBERCULOUS LESIONS FOLLOWING PLEURAL EFFUSION, CLASSIFIED BY NATURE OF LESIONS

	Number	Per Cent
Pulmonary parenchymal disease alone	49	53.2
Pulmonary parenchymal disease and pleural effusion	14	15.2
Recurrent pleural effusion alone	10	10.9
Extrathoracic and pulmonary tuberculosis	8	8.7
Extrathoracic tuberculosis and pleural effusion	6	6.4
Extrathoracic tuberculosis, pulmonary tuberculosis, and pleural effusion	4	4.3
Extrathoracic tuberculosis alone	1	1.1
Total	92	

TABLE 7

DISTRIBUTION OF INTRATHORACIC LESIONS (PULMONARY AND PLEURAL EFFUSION), WHICH DEVELOPED FOLLOWING PLEURAL EFFUSION, IN RELATION TO HEMITHORAX INVOLVED IN ORIGINAL EFFUSION

	Total	Nature of Subsequent Lesion		
		Pulmonary	Pleural Effusion	Both
Total	91*	57	16	18
Homolateral	44	33	8	3
Contralateral	32	23	7	2
Bilateral	15	1	1	13

* One recurrence of the 92 was extrathoracic spread only.

mal disease and extrathoracic spread of disease; 6 had extrathoracic tuberculosis and pleural effusion; 4 had recurrence with pulmonary parenchymal disease, pleural effusion, and extrathoracic spread of disease; finally, one had only extrathoracic spread of disease (table 6).

The distribution of intrathoracic lesions (pulmonary and pleural effusion), which developed following pleural effusion, in relation to the hemithorax originally involved was as follows: Of 57 pulmonary parenchymal recurrences, 33 were on the same side, 23 on the opposite side, and one recurrence was bilateral. Of 16 recurrent pleural effusions, 8 were on the same side, 7 on the opposite side, and one was bilateral. Of 91 intrathoracic recurrences, 44 were on the same side, 32 on the opposite side, and 15 on both sides (table 7).

Extrathoracic tuberculosis: There were 23 instances of extrathoracic tuberculosis in 19 persons, viz., one in the eye, one in the larynx, 10 lymphadenitis, one in the spine, one in the elbow, one in the knee, 2 in the gastrointestinal tract, 2 in the genitourinary tract, 2 perirectal abscesses, one miliary tuberculosis without meningitis, and one meningitis without evident miliary disease. As shown, some of the tuberculous complications which developed consisted of combined involvements. Most of the extrathoracic active disease became evident early, i.e., in the ninth to fifteenth month after onset. All of the 5 persons who died of tuberculosis developed demonstrable extrathoracic disease soon after apparent recovery from their original pleural effusion.

Time after onset: Time intervals between the onset of pleural effusion and demonstrated appearance of tuberculosis ranged widely from nine months in 13 instances to five years in 4, with an average of nineteen and four-tenths months. Thirty-eight (41.3 per cent) of the 92 reactivations appeared within the first year; 26 (more than one-quarter) in the second year; and 17 (18.5 per cent) in the third. Thus, a total of 81, almost 90 per cent of all of the 92 cases of tuberculosis, became evident within the first three years following the pleural effusion (table 8). Generally, the development of active tuberculosis occurred later in those patients who had undergone a longer period of initial treatment.

The right-hand column of table 8, under the title "Probability of Initial Re-

TABLE 8

PATIENTS WITH PLEURAL EFFUSION WHO SUBSEQUENTLY RELAPSED WITH TUBERCULOSIS, CLASSIFIED BY TIME INTERVALS IN YEARS AND BY PROBABILITY OF RELAPSE IN PER CENT

Time Interval Between Effusion and Relapse, in Years	Number Under Observation and Free of Relapse at Start of Interval	Relapsed	Probability of Initial Relapse in Per Cent	
			Annual	Cumulative*
Less than 1	141	38	27	27
1 to 2	103	26	25	45
2 to 3	77	17	22	57
3 to 4	60	7	12	62
4 to 5	53	4	8	65

* Derived by successive multiplication of probabilities of not having relapse.

lapse in Per Cent," requires explanation. The percentages represent the "corrected relapse rate" for each one-year period, i.e., the figures are based on the number of persons who were still living and well at the beginning of that year. In the first year, all of the 141 were living. The relapse cases totaled 38, the relapse rate was 26.9 per cent. In the second year, 38 of the original 141 persons already had developed tuberculosis, so that the basic group totaled only 103 persons; with 26 tuberculous cases in that year, the rate of appearance of tuberculosis was 25.3 per cent. At the beginning of the third year, 64 persons already had developed tuberculosis, leaving 77 living and well; when 17 of these developed other active tuberculosis, the rate for that year became 22.1 per cent.

It is of great interest and importance that 16 relapses in 16 persons consisted of small pulmonary infiltrates, presumably of an exudative nature, which were discovered incidentally by routine follow-up roentgenograms in asymptomatic men who were continuing their normal activities. These shadows disappeared in all instances under observation. Their tuberculous origin and activity were evidenced by the discovery of tubercle bacilli in all cases in the sputum or gastric washings. In 7 of the 16 cases, after temporary roentgenographic disappearance (regression) of the lesions, they reappeared and progressed to manifest signs and symptoms of active pulmonary tuberculosis. It is further worthy of note that numerous sputum and gastric washings had been consistently negative for tubercle bacilli before the initial appearance of these small lesions.

These observations with respect to relapse confirm what many physicians have known or suspected: That one may have active pulmonary tuberculosis and heal it without recognized or even recognizable symptoms. If the follow-up observations had not been very meticulous, these relapses would have been overlooked. The inclusion of such cases among the relapses partially explains the high rate of observed relapse after pleurisy with effusion.

Ninety-two (65.2 per cent) of the 141 patients in the study experienced relapse during the five-year follow-up period (table 1). The 92 patients with recurrent disease underwent a total of 108 separate bouts of relapse. Only 2 experienced more than two subsequent relapses in the observation period. The subsequent active tuberculosis was manifested in 133 separate organs (or types of organs) of the body in the 92 patients. The number of actual relapses (108 in 92 patients), when calculated on a patient-month basis, was 1.12 per 100 patient-months.

DISCUSSION

Because many patients with pleurisy recovered completely and remained perfectly well thereafter and because in many instances neither tubercle bacilli nor any other microorganisms were demonstrated in the pleural fluid, the tendency has been to cling to the confession-of-ignorance term "idiopathic" and to treat the condition with complacency. Acute serofibrinous pleurisy with effusion in a young adult with a positive tuberculin reaction and no demonstrable tubercle bacilli in sputum, gastric washings, or pleural fluid should be diagnosed and treated as tuberculous. A real problem arises in a similar situation when the cutaneous tuberculin reaction is repeatedly negative. It is to be emphasized that

in every one of the cases in the present study the tuberculin reaction was positive. It is exceedingly rare for the tuberculin reaction to be negative when the pleurisy is tuberculous in origin. In case of doubt, whether the tuberculin reaction has been negative or positive, a biopsy of the pleura has been revealing.

The almost immediate and apparently perfect recovery from an attack of pleurisy and the additional not infrequent occurrence (observed by Forlanini, J. B. Murphy, and Austin Flint, among many others) of the improvement[9] of an underlying pulmonary tuberculous lesion after resolution of a large effusion have long been recognized. These have led to the too often quoted but unfortunate statement that acute serofibrinous pleurisy with effusion at the onset of manifest pulmonary tuberculosis was a "happy accident."

Elimination of the 13 cases in which relapse, pulmonary and/or extrathoracic, occurred in the ninth month or later but during the original hospitalization left 128 cases of the most favorable form of acute serofibrinous pleurisy with effusion. In approximately half of these cases, the patients received less than six months of bed rest. Ninety-one per cent of the men so casually treated later experienced relapses which hardly would seem to be the behavior of a benign affair.

As the 13 relapses which occurred during hospitalization might be considered failures of rest treatment, for statistical purposes they may be included in the group which received six months or more of treatment. If this is done, the number of relapses would be 32 of 72 patients, or a 42.7 per cent relapse rate compared with a 90.9 per cent relapse in those who had less than six months, or a mean of four and eight-tenths months of treatment.

Pleurisy with effusion is not an isolated local infection, but part of a generalized infectious process. This is evidenced by the not infrequent concurrent or subsequent complication of extrathoracic tuberculosis, the experimental proof of the rapid dissemination of tubercle bacilli after infection (19), and the demonstration by liver biopsies of tuberculous lesions in the livers of three of eight young adults with acute pleurisy with effusion (20).

The cases of unresolved pleuritis and continuing signs and symptoms, both pleural and pulmonary, fall into a different category from the group here described. In them, the parenchymal lesion is evident roentgenographically or is hidden by a subacute or chronic pleuritis. In the present cases, no such parenchymal lesions were recognized, although they were sought, and lingering pleuritis was absent.

Only brief comment can be made on pathogenesis. The type of pleurisy with effusion under consideration is apparently due to the perforation, in a tuberculin-sensitive person, of a subpleural tubercle with discharge into the pleural space of living and dead tubercle bacilli and caseonecrotic material containing tuberculoprotein. Subsequent developments will depend very largely upon the "infecting dose." The perforation of a large, caseous lesion with persistent caseonecrotic communications with the pleura, with or without pneumothorax, will obviously set the stage for a more serious situation. The clinical picture will

[9] This clinical observation suggested to both Forlanini, in 1882, and Murphy, in 1898, independently, the idea of artificial pneumothorax.

therefore vary greatly with "dose," the associated pulmonary lesions, and the treatment. It is highly probable that timely bed rest and chemotherapy would greatly reduce the risk of subsequent relapse as well as minimize the gravity of the original illness.

Finally, the writers have not overlooked the possibility that some of the present cases were reported as primary pleurisy with effusion, but may have been on the basis of lymphohematogenous disease with the effusion as the first manifestation.

SUMMARY

One hundred and forty-one young male adults, otherwise quite healthy, who developed primary serofibrinous pleural effusion while in military service were observed for at least five years after onset of illness or until death. All reacted positively to intracutaneous tuberculin. None had chemotherapy for this initial illness. The probability of the subsequent appearance of active tuberculosis was much greater in those persons less than twenty-five years of age (75.3 per cent) when they first developed effusion than in those of twenty-five years or older (54.4 per cent).

The tuberculous etiology of the original effusion was proved bacteriologically in 51 patients; 33 of these later experienced relapse, and 18 remained well. Of 90 patients whose original fluids were either not examined adequately or were negative for tubercle bacilli, 59 later developed active tuberculosis in one form or another, and 31 remained well. Presumably many, if not all, of these patients originally were tuberculous. Of those without adequate bacteriologic study of pleural fluid, 71.4 per cent had a recurrence with active tuberculosis; 60.5 per cent of those with sterile fluids later developed evidence of tuberculosis.

Relapse was as frequent with small effusions as with larger ones. In no instance did a pulmonary parenchymal lesion develop later in that area which was originally obscured by the fluid.

Return to normal physical activity after *brief hospitalization* was followed by relapse with active tuberculosis in 90.9 per cent of 66 persons. Approximately 90 per cent of 92 persons who developed active tuberculosis did so within the first three years after onset of the original pleurisy. Nineteen persons developed 23 instances of extrapulmonary tuberculosis; most of the extrapulmonary disease became evident in the ninth to fifteenth month after onset of pleurisy.

The present report emphasizes the fact that, in young adults, acute serofibrinous pleurisy with effusion of the type here discussed is practically always tuberculous in origin. The pleurisy is often simply a local and dominant manifestation of a disseminated infection, if not a disseminated disease. The underlying disease is prone to relapse like all tuberculosis; hence the person with the disease must be treated accordingly.

SUMARIO

Derrame Pleural Serofibrinoso Primario en el Personal Militar

Ciento cuarenta y un varones jóvenes, en lo demás absolutamente sanos, que manifestaron derrame pleural serofibrinoso primario mientras prestaban servicio militar, fueron

mantenidos en observación a lo menos cinco años después de la iniciación de la dolencia o hasta la muerte. Todos reaccionaron positivamente a la tuberculina por vía intracutánea. Ninguno recibió quimioterapia por esa enfermedad inicial.

Las probabilidades de que sobreviniera decaimiento con tuberculosis activa fueron mucho mayores en las personas de menos de veinticinco años de edad (75.3 por ciento) al manifestar el derrame por primera vez que en las de veinticinco años o más de edad (54.4 por ciento).

La etiología tuberculosa del derrame primitivo fué comprobada bacteriológicamente en 51 sujetos; 33 de éstos experimentaron después recidivas y 13 permanecieron sanos.

En 90 enfermos, el líquido primitivo bien no fué examinado adecuadamente o resultó negativo para bacilos tuberculosos. Cincuenta y nueve de los 90 manifestaron después tuberculosis activa en una forma u otra. Los otros 31 permanecieron bien. Presuntamente, muchos de éstos, sino todos, eran tuberculosos.

De aquéllos en que no se hizo un adecuado estudio bacteriológico del líquido, 71.4 por ciento tuvieron una recurrencia de tuberculosis activa; sucedió otro tanto después con 60.5 por ciento de los que tenían líquido negativo.

Las recidivas fueron tan frecuentes con los derrames pequeños como con los grandes. En ningún caso se presentó una lesión parenquimatosa pulmonar más tarde en la zona primitivamente ensombrecida por el líquido.

El retorno a una actividad normal después de una breve hospitalización fué seguido de una recaída con tuberculosis activa en 90.9 por ciento de 66 personas. Aproximadamente 90 por ciento de 92 personas que manifestaron tuberculosis activa lo hicieron dentro de los primeros tres años consecutivos a la iniciación de la pleuresía primitiva.

Diecinueve personas mostraron 23 casos de tuberculosis extrapulmonar la mayor parte de esta afección extrapulmonar se puso de manifiesto del noveno al décimoquinto mes consecutivo a la iniciación.

Recalca esta comunicación el hecho de que, en los adultos jóvenes, la pleuresía serofibrinosa aguda con derrame de la forma aquí descrita reconoce casi siempre origen tuberculoso; es a menudo meramente una manifestación local y dominante de una infección, sino enfermedad, diseminada; es susceptible de recidivas, lo mismo que toda tuberculosis; y debe ser tratada prontamente.

RESUME

Epanchement séro-fibrineux pleural primaire chez des soldats

Cent quarante et un jeunes hommes, par ailleurs en très bon état général, qui avaient présenté un épanchement séro fibrineux pleural primaire au cours de leur service militaire, ont été suivis pendant un minimum de cinq ans après l'affection, ou jusqu'au décès. Tous présentaient une réaction positive à la tuberculine. Aucun d'entre eux n'avait été soumis à la chimiothérapie pour cette affection.

La proportion de l'éclosion d'une tuberculose en activité était beaucoup plus élevée chez les sujets qui avaient moins de vingt-cinq ans (75, 3%) quand l'épanchement s'était manifesté pour la première fois que chez ceux de vingt-cinq ans et au-dessus (54, 4%).

L'étiologie tuberculeuse de l'effusion initiale à été démontrée bactériologiquement chez 51 patients; 33 d'entre eux ont présenté des rechutes et 18 sont demeurés bien portants.

Chez 90 patients, le liquide initial n'avait pas été examiné de façon satisfaisante ou avait été BK négatif. Parmi ces 90, 59 ont présenté une tuberculose en activité, sous une forme quelconque; 31 sont demeurés bien portants. Il est probable qu'un grand nombre de ces sujets, sinon tous, étaient tuberculeux.

Parmi ceux privés d'un examen bactériologique adéquat du liquide pleural, 71, 4% ont eu une récurrence du processus tuberculeux actif; 60, 5% de ceux ayant eu un liquide "négatif" ont eu des accidents plus tard.

Les rechutes étaient aussi fréquentes avec les épanchements peu abondants qu'avec les épanchements importants. Il n'a été observé dans aucun cas l'apparition tardive d'une lésion du parenchyme pulmonaire au niveau du segment initialement obscurci par le liquide.

Le retour à une activité normale après une *brève hospitalisation* était suivi de rechute, avec tuberculose en activité dans 90,9% des cas, sur un nombre de 66 sujets. Environ 90% des 92 sujets ayant une tuberculose active l'ont présentée dans les premiers trois ans qui ont suivi la pleurésie initiale.

Chez 19 sujets, il s'est produit 23 manifestations de tuberculose extra-pulmonaire; la plupart de ces affections extra-pulmonaires se sont manifestées du neuvième au quinzième mois après l'accident initial.

Ce rapport met l'accent sur le fait que, chez l'adulte jeune, la pleurésie séro-fibrineuse aiguë avec épanchement, du type étudié ici, est pratiquement toujours d'origine tuberculeuse; elle représente souvent la manifestation locale prédominante d'une infection disséminée, sinon d'une affection généralisée; elle a une tendance aux rechutes comme toutes les formes de la tuberculose et doit être traitée promptement.

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THE PROBLEM OF MEDIASTINAL TUBERCULOSIS[1,2]

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INTRODUCTION

The purpose of this paper is to consider the clinical problems associated with tuberculous lesions of the mediastinum. The discussion will encompass: (*1*) the clinical manifestations, (*2*) the diagnostic features, and (*3*) the treatment of certain disorders due to mediastinal tuberculosis. The effects of mediastinal tuberculosis on the bronchopulmonary structures, the thoracic vascular organs, the esophagus, and the tuberculous lesions which produce no significant symptoms will be discussed. Tuberculosis of the myocardium will not be considered, since numerous authoritative articles which deal with this type of tuberculous mediastinal disease (1–10) have appeared in the literature.

GENERAL CONSIDERATIONS

Mediastinal tuberculosis produces disorders which are characterized by chronicity and by their actual or potential seriousness. Enlarged lymph nodes due to tuberculosis are of relatively common occurrence, but the harmful potentialities of these lesions have been accorded only moderate emphasis in the literature. Suppurative pulmonary disease is not an uncommon sequela of this form of tuberculosis, and it may assume various forms. Buckles (11) has emphasized the very serious nature of the disease which may be produced in the lung parenchyma as the result of bronchial obstruction due to large calcified hilar nodes. Head and Moen (12) also called attention to the mechanical effect of enlarged tuberculous lymph nodes upon the pulmonary air passages. Compression of the lumen or erosion into a bronchus by a tuberculous node may produce a variety of serious conditions, including severe hemoptysis, atelectasis, recurring pneumonia, bronchiectasis, and lung abscess. Graham, Burford, and Moyer (13) point out that bronchial stenosis may arise also from the spread of infection through the bronchial wall, with edema and eventually stricture. These writers also point out that, once lymphadenopathy has developed, the essential step in a vicious cycle has become established. Obstruction to the bronchus inevitably leads to infection, and infection perpetuates the lymphadenitis.

The relationship between primary tuberculosis and caseous lymph nodes of the mediastinum has been pointed out by many writers (11–22). The adverse effects

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of the enlarged mediastinal nodes were recognized and described in earlier medical literature (22, 23). The development of the clinical manifestations secondary to enlarged mediastinal lymph nodes is explained by consideration of their anatomic location. The regularity of the anatomic position of the hilar lymph nodes has been observed by Nelson (24). This constant relationship of the hilar nodes to the bronchial tree and pulmonary vessels, with its clinical import, has been demonstrated by several studies (12, 21, 25). The close proximity of the lymph nodes to the tracheobronchial passages accounts for the bronchial compression, constriction, or occlusion.

Head and Moen concluded from a review of the literature and their own study that there was an almost equal distribution of involvement among the bronchi to all the lobes with the exception of the right upper lobe bronchus, which was most affected (30 per cent of all cases), and the lingular bronchus, which was not affected at all. Their experience disclosed most frequent involvement of the right upper, the left upper, and middle lobe bronchi, in that order. The experience of the present writers and other reports (13, 17, 21, 26, 27) indicate that the right lung is most frequently involved. The middle lobe is mentioned in these reports as by far the most frequently affected, and the writers' experience is in accord.

The bronchus to the right middle lobe, more than any other, is subject to compression by enlarged and cicatrizing lymph nodes and is narrower than any other lobar bronchus. As a consequence, it is more easily compressed. The key node is on the inferior surface of the bronchus. As a result, the right middle lobe is particularly vulnerable to collapse. This has been such a common clinical observation that the term "middle lobe syndrome" has been used to denote the condition. At times, only a portion of the middle lobe is affected. When this is present, one of the segments is atelectatic, while its companion segment will show compensatory emphysema. In the experience of the writers, the lateral segment is the one most frequently atelectatic. Brock (21) states that, when a bronchostenosis or bronchiectasis is confined to the lateral segment of the middle lobe, it is strongly indicative of a post-tuberculous lesion. The emphysema may at times be due to partial obstruction of its segmental bronchus rather than be compensatory in nature. Meyerson (18) has stressed that obstructive emphysema may be as significant a lung-destroying process as severe pulmonary suppuration.

The effects of enlarged lymph nodes may be evidenced during the active infectious phase of primary tuberculosis or very soon after its subsidence. On the other hand, these adverse effects may be delayed until years later. When they occur at a later period, calcification may be a prominent feature in the nodes involved in the production of the bronchial compression. Buckles (11) believes that, in those cases which occur months or years after the primary tuberculous infection, secondary bacterial infection is responsible for the lymphatic enlargement. There is reason to believe that lymph nodes previously affected by tuberculosis are more susceptible to such infection. Forbus (28), in discussing granulomatous inflammation, mentions that the healing lesion, by the very nature of such inflammation, may give rise to effects which are as dangerous as the progress-

ing disease. Enlarged tuberculous lymph nodes, when healing by fibrosis, may produce bronchial compression by cicatricial encirclement of a bronchus. This may be the only process which causes delayed complications.

In children with primary tuberculosis, the lymph nodes often become greatly enlarged and may produce marked bronchial compression. They may cause partial atelectasis, a condition which was formerly termed "epituberculosis" (29, 30). It frequently occurs in the right upper lobe. The recognition of the condition as atelectasis due to bronchial obstruction by enlarged lymph nodes has been repeatedly stressed (14, 25, 27, 31, 32). A typical case of this type of obstruction due to primary involvement of the lymph nodes with tuberculosis is illustrated by the clinical course of the patient in *Case 5 (see* Plate I). It also demonstrates how the condition may be mistaken for a bacterial inflammatory process.

Although the lobe appears normal upon subsidence of the enlargement of the lymph nodes and reopening of the bronchus, the condition is not always benign. Chronic nontuberculous bronchiectasis in the involved area may result. The lymph node enlargement is ordinarily due to a primary tuberculous lesion, but it may also result from a hematogenous-lymphogenous spread of tuberculous disease.

Extrinsic pressure on the bronchus may not be the only cause of obstruction; actual erosion into the lumen of the bronchus is sometimes the etiologic factor. This erosion may be due to a caseous lymph node or to a calcified focus in a mediastinal node (16, 18, 21, 32). Other related causes of bronchial occlusion are endobronchial ulcerations without erosion or intraluminal tuberculous granulation tissue. Ulceration of the bronchial mucosa is often due to the contiguity of an enlarged infected lymph node (18, 32).

Bronchial obstruction from any of the factors mentioned is the primary factor in producing subsequent suppurative disease, especially bronchiectasis. This cause and effect relationship has been fully established by pathologic studies (32, 33). The extent of the suppurative disease will obviously vary from case to case, depending upon the site of the obstruction, the degree of the occlusion, and the extent and character of the distal infection. An example of the varied clinical picture which may be presented by such obstruction is exhibited in *Case 1* as follows:

Case 1. A 26-year-old Marine sergeant was admitted to the hospital because of routine chest roentgenographic findings of an abnormal density in the right hilar region. On history it was found that a cough was present for the past week. This was productive of approximately 20 cc. of whitish sputum. There were no other symptoms or any significant family history, even after repeated questioning.

Physical examination disclosed no abnormalities. Temperature was 98°F.; pulse, 96 per minute; respiration, 20 per minute; blood pressure, 120 systolic, 84 diastolic in mm. of mercury.

The chest roentgenograms showed right hilar enlargement (figure 1). The total leukocyte count was 4,500 per cu. mm., with 72 per cent polymorphonuclears, 26 per cent lymphocytes, 2 per cent eosinophils. The erythrocyte sedimentation rate was 2 mm. Cutaneous reaction to tuberculin, first-strength PPD, was negative and, to second-strength PPD, was 2-plus.

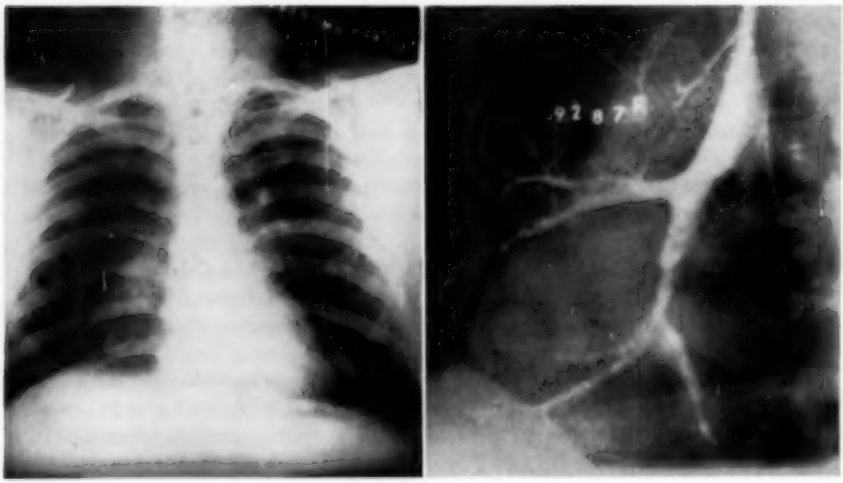

FIG. 1. *Case 1.*

(Left) Postero-anterior view of the chest roentgenogram showing right hilar enlargements.

(Right) An enlarged view of bronchography demonstrating the absence of filling of the middle lobe and the stenosis of the posterior medial segmental bronchus of the right lower lobe.

Histoplasmin and coccidioidin skin reactions were negative. Albumin and globulin protein concentrations were normal.

The course in the hospital was afebrile and asymptomatic. Bronchoscopy showed some questionable narrowing of the middle lobe orifice. Bronchography on two occasions failed to show filling of the middle lobe and demonstrated a stenosis of the midportion of the posterior medial segment of the lower lobe bronchus. Further roentgenographic studies with plain frontal, lateral, and Bucky plates showed that the middle lobe was atelectactic. With these findings the right hilar region remained enlarged.

Repeated studies of bronchial aspirates and sputum were negative for bacteria, fungi, and tubercle bacilli. Papanicolaou studies of neoplastic cells were negative. Exploratory thoracotomy showed dense fibrous inflammatory tissue involving the mediastinal hilar nodes and encircling the middle lobe bronchus and the lower lobe bronchus. Because of the persistence of the abnormalities, surgical exploration was deemed advisable. Dissection of the hilar structures and the vessels was extremely difficult. It was also noted that nodular lesions were present throughout the lower lobes and the middle lobe. Pathologic examination of the tissue removed revealed tuberculosis of the lymph nodes. Tuberculosis of the bronchi was noted, tubercles were present throughout the submucosal areas, and in several places the tubercles were actually elevating the mucosa. Some caseating tubercles were scattered throughout the pulmonary parenchyma. One small pulmonary infarct was noted in one location in the posterior basal segment of the lower lobe.

The postoperative course was uneventful. The patient is now receiving strict bed rest and streptomycin-para-aminosalicylic acid (PAS) therapy. At the last report he was progressing satisfactorily without recurrence of his disease.

Further examples of the varied effects of mediastinal tuberculosis producing pressure on the tracheobronchial passages are illustrated by *Case 2*, which fol-

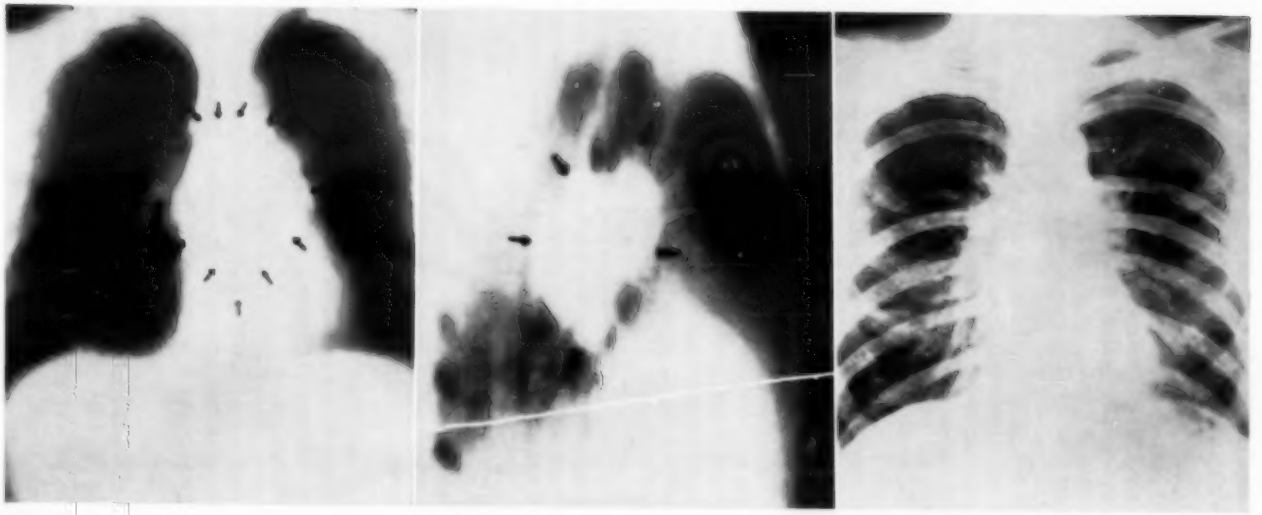

FIG. 2. *Case 2.*

(Left) Antero-posterior tomographic cut showing the large mass outlined by arrows in the posterior mediastinum.
(Center) Lateral tomogram showing the large mediastinal mass outlined by arrows.
(Right) Postero-anterior chest roentgenogram showing the appearance after the removal of the mass.

lows, and by *Case 6* (*see* Plate II). In the one patient there is a more quiescent process as compared with an active process in the other patient.

Case 2. A 35-year-old naval officer was asymptomatic but, on routine roentgenography, a mass was disclosed in the posterior mediastinum. The past history included frequent colds, a chronic nonproductive cough, and an 8-pound weight loss in the preceding year. For the past month dyspnea on exertion had been noted. The past and family histories and systemic reviews were noncontributory.

The physical examination was normal. Venous pressure studies were reported as 500 mm. of water on the right and 550 mm. of water on the left. The circulation time was delayed on the right (decholin), being 30 seconds, and 17 seconds on the left. The chest roentgenograms disclosed a large mass in the posterior subcarinal region of the mediastinum. It measured 6 by 10 cm. A barium esophagram showed the mediastinum to be displaced backward by this mass. Gastrointestinal and pyelographic studies were normal. The skin reaction to tuberculin (second-strength PPD) was positive. Cultures of gastric aspirate for *Mycobacterium tuberculosis* were negative. Other systemic roentgenographic examinations, laboratory studies, and other skin tests were noncontributory.

Bronchoscopy showed only widening of the carina. An angiocardiographic examination was normal.

The tomographic studies showed that the lesion was located in the right posterior mediastinum and extended 1 cm. into the left lung field.

A thoracotomy was performed, and a stony hard tumor mass was found projecting from the right mediastinum. The superior pole of the mass was at the level of the inferior pulmonary vein. The adjacent structures were tenaciously adherent to the mass. The azygos vein and periphery of lung were freed. The mass was solidly fused with the esophagus, but after tedious dissection it was freed. It was also difficult, because of adhesions, to free the lower pole. The mass was densely adherent to the posterior membranous wall of the right main stem bronchus and carinal region. At the superior pole a portion extended posterior to the trachea and passed around the carina up anteriorly to the trachea. It was fused to the posterior wall of the left main stem bronchus as it had been on the right. Furthermore, it was fused to the wall of the aorta. The right vagus passed deep into the tumor and had to be sacrificed. A left thoracotomy was done twenty-two days later; the rock-hard tumor mass was encountered. It was densely adherent to the thoracic aorta for a distance of 6 cm., and the first portion of the descending aorta was adherent to the inferior pulmonary vein. By tedious blunt dissection, it was finally freed.

At the left main stem bronchus, the mass was also densely adherent. Rents were made in both the bronchus and the trachea, but were successfully closed. The tumor was then removed *in toto* from the chest.

The postoperative condition was good. The patient was out of bed on the third day. A postoperative bronchoscopy one month later was normal except for an extra ridge at the carina and slight narrowing of the left main stem bronchus.

He was well, and two months later was returned to duty. At present, he is asymptomatic and performs complete military duty.

The pathologic examination showed multiple cystic areas containing caseation. The cyst walls contained a great deal of calcification and were surrounded by dense fibrous tissue. Foci of epithelioid and granulomatous-type giant cells were seen. This was considered to be consistent with tuberculosis.

DIAGNOSIS

The symptoms in the patients with tuberculosis of the mediastinum may assume one or more of the following forms: *chronic cough*, *recurrent hemoptyses*, *general debility with or without associated chest pain*, *a single acute pneumonic episode*, *recurrent febrile episodes*, or *a clinical picture simulating bronchogenic carcinoma*.

Clinical manifestations: There are certain clinical characteristics which may prove helpful in the diagnosis of suppurative disease secondary to mediastinal tuberculosis. The sputum of persons so affected is greenish or yellowish and often not as purulent or odoriferous as in the usual case of bronchiectasis. The cough is usually chronic, more often moderate, but at times may be spasmodic, croupy, or wheezy. A history of chest pain is common. The presence of an antecedent pleural effusion is helpful in suggesting tuberculosis. In children, more often than in adults, there is an associated localized "wheeze." This must be differentiated from bronchial asthma with its generalized sibilant rales. A low-grade fever is occasionally present, but fever is commonly noted only during an acute exacerbation of bacterial infection. In children, however, the temperature may be elevated throughout the course. Hemoptysis is a frequent symptom. It may be the presenting symptom and is often recurrent. Bleeding may be profuse. Calcified particles (broncholiths or "stones") may be expectorated (34). Direct questioning is sometimes necessary to elicit a history of such an occurrence. Often the patient will save the stones which have been coughed up. Rarely a piece of material may be expectorated which represents a fragment of caseous lymphoid tissue. The presence of carbon particles in the sputum is considered significant of tuberculous lymph node erosion of a bronchus (35).

Roentgenography: Roentgenographic examination of the chest is of paramount importance, and the findings usually strongly suggest the nature of the disease. The customary postero-anterior and lateral views will suffice to disclose the presence of atelectasis, either partial or complete, and will show whether it is lobar or segmental in extent. Emphysema may also be demonstrated. When the middle lobe is involved, however, special roentgenographic techniques should be utilized. A lordotic position is helpful in the demonstration of one or both borders of the middle lobe. This view will exclude simple enlargement of the hilar lymph nodes, pleuropericardial adhesions, or an interlobar effusion. The last-named condition is encountered rarely and, when present, will be associated with either parenchymal pulmonary disease near the middle lobe or congestive heart failure.

The roentgenographic criteria for distinguishing between atelectasis of the middle lobe and interlobar effusion have been described by various writers (36–41). Further special roentgenographic techniques are necessary occasionally for the identification and accurate localization of the pulmonary lobes or segments involved. The presence of calcification in the hilar lymph nodes is of definite value in directing attention to tuberculosis. Tomography, overexposed Bucky films, inspiratory and expiratory roentgenograms, and other special views may be indicated. The special techniques of roentgenography, fluoroscopy, physical diagnosis, and skin tests which may assist in the diagnosis of bronchial obstruction have been fully described in other papers (17, 36, 40) and will not be considered in detail.

Bronchoscopy: Bronchoscopy should be done in every case. It is indicated for diagnostic purposes and may be of therapeutic value as well. The findings at bronchoscopy are often normal, but the presence of any of the following findings will aid in the diagnosis and localization of the disease: ulceration of a bronchus,

an area of perforation, narrowing due to extrinsic pressure, chondromalacia, intraluminal granulation tissue, or pus exuding from a particular bronchus.

In the absence of such observations, the presence of anthracotic pigment in the bronchial mucosa, particularly in the region of compression, is regarded as circumstantial evidence of perforation by a lymph node (35). In children, bronchoscopy has also been advocated for the purposes of therapy (16, 21).

Bronchography: Bronchography is frequently indicated. In some clinics it is recommended as a routine procedure (12, 18). Buckles (11) believes that bronchography is of the greatest value in localizing the lesion. One of the recently developed, quickly soluble, opaque media should be employed to avoid the obscuration of the pulmonary fields which is produced by residual Lipiodol®. With the use of this technique, it is usually possible to demonstrate clearly any significant narrowing of the bronchus beyond the vision of the bronchoscopist.

Differential diagnosis: The differential diagnosis is made difficult by the fact that the secondary changes in the pulmonary parenchyma overshadow and obscure the primary mediastinal lesion. A *foreign body* in the tracheobronchial tree presents features which clinically and roentgenographically resemble the sequelae of mediastinal tuberculosis. Failure to visualize a foreign body at bronchoscopy and the observation of extrinsic pressure on the bronchus will point to the condition as extrabronchial. Overexposed roentgenograms may disclose the shadow of a foreign body. The presence of calcium in the hilar lymph nodes is a clue to tuberculosis.

A *bronchogenic carcinoma* may be suggested in older patients by hemoptysis, the absence of significant bacteriologic findings on examination of the sputum, or by recurrent pneumonia, atelectasis, or pulmonary suppuration. Papanicolaou stains of the sputum or bronchial aspirate for neoplastic cells may be helpful. The evidence is conclusive if such stains show malignant cells. Biopsy material obtained by bronchoscopy, if positive for neoplastic disease, obviously will solve the diagnostic problem. In his report of eight cases of mediastinal tuberculosis, Buckles (11) mentions a definite preoperative diagnosis of bronchogenic carcinoma in one and a probable diagnosis of malignancy in three other cases. Brock (21) also cites carcinoma as deserving outstanding consideration in the differential diagnosis of pulmonary lesions secondary to mediastinal tuberculosis.

Bronchial adenoma may be suspected in persons who present the symptoms commonly due to mediastinal tuberculosis. *Bronchiectasis* is suggested by the array of clinical manifestations. The character of the sputum, when considered with the nature of the cough, may aid in differentiating between the two conditions. However, bronchiectasis may very well be present as a sequela to bronchial obstruction secondary to mediastinal tuberculosis. The bronchiectasis per se then becomes the all-important therapeutic consideration. The presence or absence of enlarged tuberculous mediastinal lymph nodes becomes a matter of academic interest, except as an explanation of the etiologic basis of the significant disease. *Cholesterol* pneumonitis may simulate the disease under consideration, but the absence of bronchial obstruction is of diagnostic import. This is true even though hemoptysis, enlarged hilar lymph nodes, atelectasis, and chronic

pneumonitis of lobar or segmental distribution may be present in both disorders. *Primary pulmonary abscess* offers a minimum of difficulty in differential diagnosis, but secondary abscess formation can occur as a result of bronchial obstruction by enlarged tuberculous nodes.

The history is of paramount importance in the diagnosis of lung abscess. The patients with lung abscess are clinically ill, usually acutely so, as is illustrated by *Case 3*, which follows.

Case 3. A 34-year-old Negro male complained of fever and chills. He was completely well until three months before admission. He became ill with a cold, cough, and substernal tightness in his chest. The cough was productive of one-half cup of yellowish sputum a day. Five weeks before admission the fever and chills developed. At that time he attended a clinic where, after examination and a chest roentgenogram, he was advised to return home. One week later, he returned to the same clinic, where, because mediastinal enlargement was now noted, he was referred for hospitalization. His symptoms continued. The night before admission he had a profuse sweat. His sputum was approximately 50 cc. in amount each day. It was purulent in nature, nonfoul, and had never contained blood.

The physical examination disclosed an expiratory wheeze on forced expiration over the right middle lobe area. A few fine rales were heard over the right lower lobe. He was acutely ill. His temperature was 99°F., the pulse rate was 90 per minute, and his blood pressure was 110 systolic, 70 diastolic in mm. of mercury. The remainder of the physical examination was unrevealing.

The laboratory studies were reported as follows: the blood counts and urinalysis were normal; the tuberculin skin reaction was positive to first-strength PPD. Skin tests to histoplasmin and coccidioidin were negative. Repeated studies of the sputum and gastric washings were negative for all pathogenic organisms, including tubercle bacilli. The chest roentgenogram taken in the clinic on the patient's first visit war normal. The admission roentgenographic study showed a right hilar mass and blunting of the right costophrenic sinus. The entire right lung appeared less aerated than the left (figure 3, upper left).

Esophageal barium studies showed a traction diverticulum of the upper third located just above the aortic arch.

The hospital course was characterized by a septic course, with profuse sweating and daily temperature elevation to 103 or 104°F. Wheeze over the right middle lobe became increasingly prominent. A pleural friction rub developed and there were increasing rales over the right lower lobe. The chest roentgenograms showed the development of consolidation in the apex of the right lower lobe and a pneumonitis of the lower lobe. Later there was breakdown in the apex of the lower lobe with a fluid-containing cavity (figure 3).

Bronchoscopic examinations showed stenosis of the right main stem bronchus, with foul smelling grayish purulent secretions coming from the lower lobe and dorsal ramus orifices.

There was an absence of response to penicillin, streptomycin, and PAS. Repeated cultures of sputum and bronchial and gastric aspirates for fungi, bacteria, and tubercle bacilli failed to reveal any pathogenic microorganisms.

An exploratory thoracotomy was performed. The parietal pleura was studded with tubercles. The middle lobe and right lower lobes were involved with bronchiectasis; the apex of the lower lobe contained a large abscess cavity which extended into the chest wall.

The postoperative course was unsatisfactory, and the patient died one week after the thoracotomy. Pathologic examination showed a putrid abscess of the apex of the lower lobe extending into the chest wall and extensive suppurative bronchiectasis of the lower and middle lobes. The hilar and mediastinal lymph nodes were enlarged and were involved in an extensive thick granulomatous reaction involving all structures of the hilum and mediastinum. The nodes were definitely tuberculous. There were some scattered areas of caseous tuberculosis in the lungs, but the main pulmonary disease was suppurative and had originated from the encroachment on the bronchi and interference with drainage.

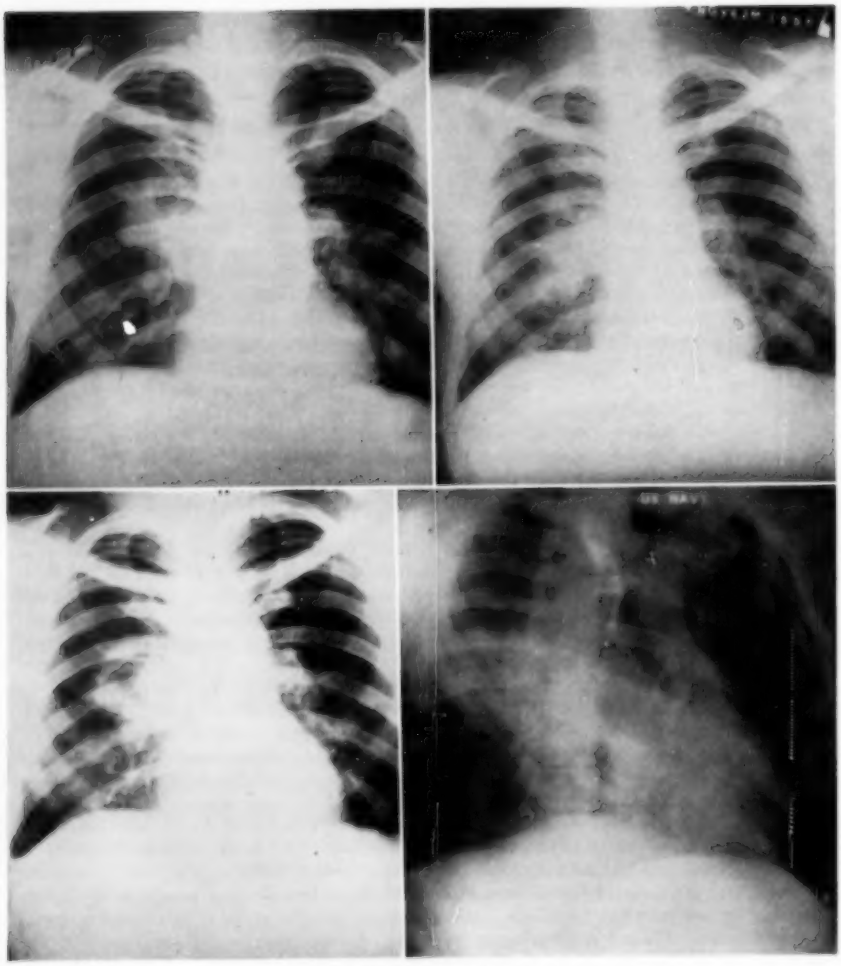

FIG. 3. *Case 3.*

(Upper left) Admission postero-anterior roentgenogram showing the right hilar enlargement and decreased aeration of the right lung field.

(Upper right) Chest roentgenogram of the same view taken three weeks later, demonstrating the increase in the hilar shadow and some blunting of the right costophrenic sinus.

(Lower left) The roentgenogram now shows definite cavity formation located in the apex of the right lower lobe.

(Lower right) A right oblique view showing the cavity located in the peripheral apex of the lower lobe on the right and the hilar and lower lobe involvement.

This patient's case is presented chiefly as an example of a suppurative putrid pulmonary abscess. Nevertheless, the primary problem was a consequence of mediastinal tuberculosis which had produced bronchial obstruction and incomplete bronchopulmonary drainage.

Wheezing and sibilant rales may be mistaken for *bronchial asthma*. The clinical history, the course of the illness, and the evidence obtained on physical examination and from the chest roentgenograms will enable one to distinguish between the two conditions. The diagnosis will be established if, in conjunction with the clinical picture, cultures positive for *Mycobacterium tuberculosis* are obtained. Such a bacteriologic finding is not made, however, in all instances.

Treatment: Secondary pulmonary disease due to mediastinal tuberculosis usually requires prompt treatment. In some cases, especially in children, bed rest and antimicrobial therapy (streptomycin, PAS, or isoniazid), administered according to some accepted regimen, will resolve the inflammatory process and lead to complete subsidence of the clinical condition (16, 42). Bronchoscopic removal of caseous material may be of great benefit. Only clinical judgment and experience will aid in the determination of the need and time for surgical therapy. The evil sequelae of long-standing progressive compression of the bronchus, such as bronchiectasis and lung abscess, definitely require surgical treatment. The aftermath of primary tuberculous lymphadenitis requires careful and prolonged observation to determine whether surgery will be necessary. Many cases of "epituberculosis" clear without damage or residue. Brock considers it unjustified to operate in the presence of active tuberculous adenitis because of the possibility of dissemination, fistula formation, and pyothorax.

Lobectomy or segmental resection are the most common procedures used for secondary suppurative lesions. At operation the pathologic lymph nodes also are removed. Hemoptysis may be sufficiently massive to require emergency treatment (12, 43).

EFFECT ON THE MEDIASTINAL VASCULAR STRUCTURES

Tuberculosis of the mediastinal nodes may produce effects on the mediastinal vascular structures with serious sequelae. The etiologic role of tuberculous lesions in mediastinal vascular disorders has not been emphasized. It is natural that caval obstruction due to neoplasm has been given considerable attention, for this may be an early symptom when the causative lesion is difficult to demonstrate. Vascular disorders also occur with tuberculosis of the mediastinum. Compression and displacement of the solid organs and obstruction of hollow viscera and blood vessels may be such prominent features that the clinical observer is diverted from the more inconspicuous aspects of the granulomatous lesion.

The effects of granulomatous lesions on vascular structures have been pointed out by several authorities (44, 53). Obstruction of the superior vena cava or innominate veins by lesions in or adjacent to the upper mediastinum has been noted. This is commonly referred to as the *superior vena caval syndrome* (44, 47, 57). Few cases of this syndrome due to enlarged tuberculous granulomas of the mediastinum have been reported. Descriptions of cases attributed to tuberculosis

appear principally in the older literature, and there is no doubt that at present neoplasm is a far more frequent cause. Little has been written within the past three decades dealing with the relationship of tuberculosis to these obstructive disorders of blood vessels. Yet, if a tuberculous lesion is rare, it remains of great importance because proper therapy can give an excellent prognosis, while treatment of the disease as cancer may hasten death.

Certain anatomic considerations serve to explain the pathogenesis of this manifestation of tuberculosis of the mediastinum. The large mediastinal veins are thin-walled, and blood flowing through them is under very low pressure. Furthermore, these veins are situated anteriorly nearer the unyielding thoracic cage, against which they may be compressed by expanding mediastinal lesions. Because these veins are easily compressed and drain blood from the upper half of the body (head, neck, and thoracic wall), obstruction to the flow of blood in the veins produces localized symptoms and pathognomonic signs of a mediastinal lesion. These manifestations may be the first indications of serious mediastinal disease.

The right anterior mediastinal and right paratracheal nodes are intimately associated with the superior vena cava. The right bronchial lymph nodes and some of the nodes at the tracheal bifurcation are in close approximation to the lower portion of the superior vena cava. Another factor of importance is that most of the structures of the left thoracic cavity drain into either the right anterior mediastinal or the right paratracheal lymph node chain, or into both. Inflammatory disease in any part of the body drained by these nodes with their subsequent enlargement may produce partial or complete obstruction of the superior vena cava or the innominate veins.

The symptoms which result are dependent upon the extent and degree of the indirect involvement of the vascular channels in the mediastinum. The effect on the vessel may be: pressure displacement, constriction with partial obstruction, or complete obstruction. The vascular obstructive symptoms must be divorced from those resulting from the granulomatous inflammation per se and from those due to involvement of other mediastinal structures. Dyspnea may be present because of interference with the return flow of blood to the right auricle. Edema and pain of the upper extremities occur later, while engorged peripheral veins are seen earlier. These findings may be unilateral or bilateral, depending upon the degree and site of vessel involvement. If the innominate veins are affected, various degrees of cervical venous engorgement and edema of the neck, face, and scalp may be seen. The presence of a Stokes' collar has never been noted with tuberculosis of the mediastinum, although it occurs frequently with lymphoblastoma and other mediastinal neoplasms. Dilated veins of the thoracic wall point to the presence of superior vena caval obstruction. Hussey, Katz, and Yater (48) have described signs and symptoms similar to those mentioned. In addition they have noted cyanosis, cough, and generalized or Jacksonian convulsions.

Obstruction of the superior vena cava is confirmed by measurement of the venous pressures in the larger peripheral veins and a study of the dilated collateral veins if any are present. The study of the venous pressures in demonstrating

venous obstruction has been discussed by numerous writers (44, 50, 59). The pressures in a brachial vein are measured by a water manometer. The zero level must be kept at 10 cm. above the level of the spine. If the pressure is found to be elevated in the veins of the arms, head, neck, and the chest above the level of the diaphragm, the obstruction is in the superior vena cava. At the same time the venous pressure in the tributaries of the inferior vena cava will be normal. They will be moderately elevated bilaterally in the inferior epigastric veins because of collateral circulation.

With obstruction to the superior vena cava, it is possible to demonstrate reversal of flow in the veins of the anterior thoracic wall. When the superior vena cava is normally patent and the pressure in the veins of the upper portion of the chest is normal, the direction of the blood flow is cephalad with the patient supine. When the superior vena cava is obstructed, the pressure is elevated in the veins of the chest, and the direction of the blood flow is caudad; i.e., the flow is through the collateral venous system into the patent inferior vena cava and thence back to the heart.

Clenching of the fists tightly several times will produce a much greater elevation in the venous pressure in the medial basilic vein on the obstructed side than on the unobstructed side with similar exercise. The present writers use this test routinely and have found it to be of particular value in differentiating venous obstruction from lymphatic obstruction and in detecting a partial obstruction. An estimate of the degree of obstruction can be made from the degree of the venous hypertension. This is commonly directly proportional to the completeness of caval obstruction, but a lesser elevation of venous pressure may be present with a greater development of collateral circulation. The circulation time may be prolonged when obstruction is present, but it is often normal and gives no conclusive aid in establishing the presence of an obstruction. Observation of the respiratory variation in the venous pressure during this examination will be of importance. Normally the venous pressure drops during inspiration, while with obstruction and a return flow through collateral circulation, the venous pressure variation is paradoxical, rising during inspiration. This occurs when the obstruction lies below the entrance of the azygos vein into the superior vena cava.

The collateral circulation may be visualized by infrared photography. Red glasses may be used by the clinical observer to note them without awaiting photography (60).

Angiography of the venous circulation will confirm and exactly localize the site of obstruction. This technique permits precise visualization of the venous involvement. The dilated collateral vessels are usually well demonstrated by the method. Neo-Iopax[8] or Diodrast[8] may be employed as the contrast medium (61). Steinberg and Dotter (62, 63) and Sussman (64) have employed this technique extensively for clarifying and providing additional information in many forms of pulmonary disease and especially in the study of mediastinal lesions.

The dangerous effects of tuberculosis of the mediastinum on the vascular structures have been emphasized by Edwards and Burchell (65) in their report of a dramatic case of acquired pulmonary venous obstruction. A granulomatous

mass was present below the tracheal bifurcation in the posterior mediastinum which caused venous obstruction of an unequal degree in the different pulmonary veins, with extreme pulmonary congestion which eventually resulted in the death of the patient.

When the diagnosis of superior vena caval or other mediastinal venous obstruction has been established, its cause must be determined. The treatment of this vascular syndrome is directed at the primary cause, be it tuberculosis, carcinoma, or lymphoblastoma. When the lesion is of tuberculous origin, the use of isoniazid or streptomycin-PAS may resolve the process (42). However, this form of treatment, especially if the diagnosis cannot be established first, must be done in conjunction with surgery.

An example of superior vena caval obstruction by tuberculous mediastinal lymph node involvement is well illustrated by the patient presented in Plate III. The excellent response that resulted from antituberculous therapy will be noted. This type of involvement may easily be confused as being due to lymphoblastoma or bronchogenic neoplasm.

Removal of the mass surgically may relieve the obstruction. If not, an anastomotic procedure on the superior vena cava or other vein may be needed after extirpation of the mass of granulomatous inflammatory tissue and the thrombosed segment of vein.

It is evident that some reported cases of this disorder have not been recognized to be due to tuberculosis. When the cases of McIntire and Sykes (55) are examined, it appears likely that many, if not all, of the sixteen cases of idiopathic etiology were actually due to tuberculosis. All of the patients had a history of previous respiratory infections months or years before their vascular obstructive symptoms, and all of the pathologic material was granulomatous in nature and contained calcium.

The present review article is based on cases studied from 1904 to 1946. Fifteen and four-tenths per cent of the cases of superior vena caval obstruction reported between 1904 and 1936 were considered to be due to chronic nonspecific mediastinitis; but subsequently (from 1936 to 1946) 54 per cent were ascribed to tuberculosis. In the latter group, only 11.7 per cent of the cases were considered of idiopathic origin. This indicates the occurrence of more precise pathologic diagnoses. Hussey (51) has described cases which were definitely of tuberculous origin and others in which the etiology was strongly suggested.

EFFECT ON THE ESOPHAGUS

The esophagus may also be involved by tuberculosis. Most often there is a fibrous attachment to the esophagus. This was noted in many of the reported cases (66, 67). The involvement occasionally consists of actual extension of the granulomatous inflammation into the esophageal wall or even perforation of the esophagus, with subsequent fistula formation (12, 68, 70). Esophagobronchial fistula through a tuberculous esophageal traction diverticulum has been reported (69–71). The association of esophageal diverticula, especially those of the midportion, with tuberculous mediastinal and bronchial lymph nodes has

been described frequently (68, 72–75). The presence of traction diverticula of the esophagus in a patient with "middle lobe syndrome" has recently been noted (76), and the writers have seen such a diverticulum in a case of constrictive pericarditis. In the majority of the writers' cases, a fibrous attachment to the esophagus was the principal finding. The attachments were usually located at the notch formed by the junction of the azygos vein and the superior vena cava. A traction diverticulum of the esophagus just above the diaphragmatic foramen was found in one case. At operation, a second patient was found to have a huge tuberculous mass located below the bifurcation of the trachea and firmly attached posteriorly to the esophagus.

The diverticula result from an extension of the inflammatory reaction to the esophageal wall with formation of dense fibrous adhesions between the lymph nodes, the peri-esophageal tissues, and the anterolateral wall of the esophagus. As a result of the contraction of the scar tissue, retraction and out-pocketing of the esophageal wall occur.

Such esophageal lesions are frequently noted at autopsy or at operation without record of previous clinical symptoms. Symptoms, if present, are usually minimal in degree. Obstruction of the esophagus is produced only when the extrinsic lesion is extremely constrictive and produces motor dysfunction. Severe hemoptysis may occur with fistula formation (69).

The presence of a diverticulum may be overlooked if the examination of the esophagus is not satisfactorily performed. The esophagus should be fully distended with a thick *barium* mixture, and the roentgenograms should be taken with the patient in a prone position. In the standing position the barium may traverse the esophagus without entering the diverticulum, or it may remain in the pouch for only a short time, thus hampering visualization and delineation of the lesion.

Preoperative recognition of the presence of an associated diverticulum is essential, for it may be firmly matted together with a diseased bronchus and adjacent lymph nodes, thus exposing it to accidental surgical trauma unless its presence has been demonstrated prior to operation. Only if the diverticulum is large and symptom-producing should consideration be given its correction. If a fistula is present, surgical treatment is mandatory. Appropriate antimicrobial therapy should be administered pre- and postoperatively. Two examples of esophageal diverticula are illustrated in Plates IV and V. One patient had substernal symptoms and difficulty in eating solid food; the other patient was asymptomatic.

EFFECT ON THE HEART AND PERICARDIUM

It has been well established that involvement of the pericardium by tuberculosis occurs by contiguity (7–10). It occasionally may result from retrograde lymphatic extension. It rarely occurs as a manifestation of miliary dissemination. It is found in 3 to 7 per cent of all tuberculous patients examined post mortem.

The presence of pericardial involvement is sometimes difficult to diagnose, for typical clinical signs are not always found. Special diagnostic procedures are often needed and many times are indicated. These helpful aids include electrokymography, angiocardiography, and cardiac catheterization.

It is accepted generally that the proper treatment of chronic constrictive pericarditis is pericardiectomy. Acute tuberculous pericarditis requires first the usual methods of antituberculous chemotherapy, especially with the inclusion of isoniazid, bed rest, and careful observation of progression or regression of the pericarditis or the lack of response. It is also of importance whether constrictive signs develop. A period of three to six months should allow a decision whether pericardiectomy should be done. It should be remembered that, the earlier pericardiectomy is performed, the easier the surgical procedure and also the less the subsequent myocardial involvement with fibrosis. Each case will require individual clinical judgment. The results have been excellent with this plan of management.

The following case summary illustrates a rather typical case of tuberculous pericardial effusion with an excellent outcome. Patients with constrictive pericarditis are not always as easily diagnosed.

Case 4. A 20-year-old Negro male was admitted to the hospital with the complaint of tightness in the mid-epigastrium of five days' duration. The patient had a poor appetite and mild malaise. There was nothing else significant in the history other than the fact that he had been living with his brother who had active pulmonary tuberculosis.

The physical examination findings were as follows: the temperature was 102°F.; pulse rate, 96 per minute; respirations, 26 per minute; the blood pressure was 122 systolic, 84 diastolic in mm. of mercury. The head, neck, and chest findings were normal. The cardiac examination disclosed a pericardial friction rub, with a systolic gallop which was heard best in the mitral and tricuspid areas. The laboratory studies were reported as follows: hemoglobin, 9.5 gm.; total leukocyte count, 5,500 per cu. mm., with a differential count of 78 per cent polymorphonuclear cells and 22 per cent lymphocytes. The electrocardiogram showed elevation of the ST segments in the standard limb leads and precordial leads. The findings were interpreted as providing evidence of pericarditis.

The chest roentgenogram (figure 4, left) demonstrated a widened cardiac silhouette suggestive of pericardial effusion.

The patient was started on streptomycin-PAS therapy. His symptoms had disappeared by the second month of treatment. By the sixth month the chest roentgenogram (figure 4, center) and electrocardiogram were normal.

Other studies, including intravenous pyelograms and osseous roentgenograms, were normal. An angiocardiographic study obtained during the ninth month of therapy disclosed no abnormalities.

A further chest roentgenogram (figure 4, right) was normal except for some slight infiltrative lesions noted in basilar segments of the left lung in the retrocardiac area and only seen on the left lateral. All gastric cultures were negative for *Mycobacterium tuberculosis*. The patient is without clinical evidence of disease and is to continue on chemotherapy for at least one year. His roentgenograms show that the process is apparently stable.

Tuberculosis of the myocardium or endocardium will not be considered.

ASYMPTOMATIC TUBERCULOSIS OF THE MEDIASTINUM

Tuberculomas of the mediastinum are often asymptomatic. These lesions have been adequately described in other papers (66, 67), and their usual location has been mentioned in the discussion of tuberculous lesions affecting the esophagus. Most of these lesions are spherical or oval in shape, smooth in contour, and of homogeneous density. They can be accurately diagnosed only by surgical explora-

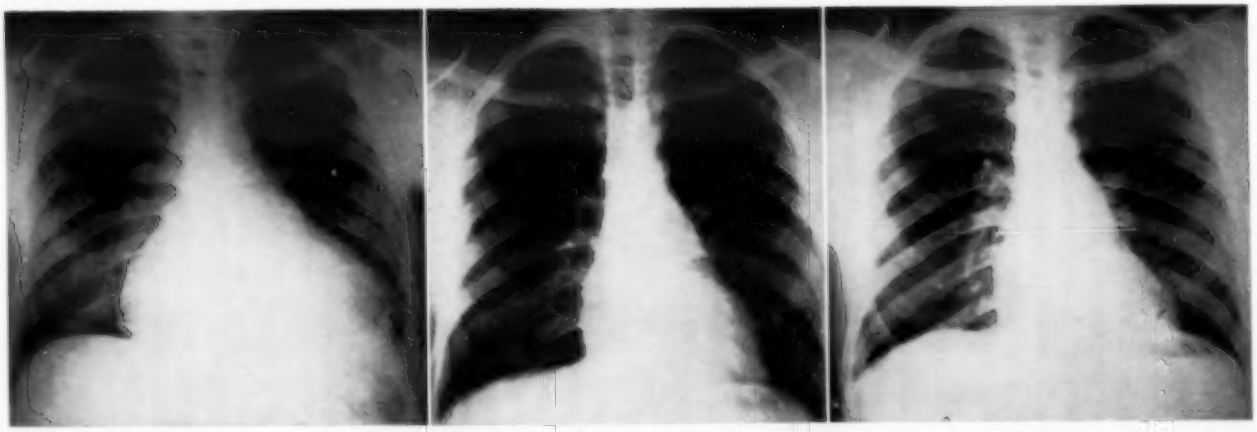

FIG. 4. *Case 4.*

(Left) Postero-anterior roentgenogram of the chest showing the pericardial effusion.
(Center) Normal cardiac silhouette seen in the postero-anterior chest roentgenogram after seven months of therapy.
(Right) Unchanged chest roentgenogram one year after previous film.

tion, and extirpation is the treatment of choice. The removal of a tuberculoma renders less likely the possibility of the development of an active tuberculous infection. The presumptive diagnosis of these masses as mediastinal tuberculomas depends on the presence of a mass of the singular size, position, and roentgenographic appearance characteristic of such lesions. A history of tuberculosis, the presence of other evidence of that disease, and a positive tuberculin reaction would strengthen the possibility of the lesion's being a tuberculous granuloma. The definitive diagnosis depends upon histologic and cultural study of the excised mass. A typical example of asymptomatic tuberculosis of the mediastinum is illustrated in Plate VI.

GENERAL COMMENT

Involvement of the mediastinum with tuberculosis can produce effects which are secondary and which often present a confusing clinical picture unless the primary cause, tuberculosis, is considered and attempts are made to discover its presence. The clinical appearance produced by mediastinal tuberculosis depends upon which of the anatomic structures may be involved by the tuberculous process. Certain anatomic relationships in the mediastinum allow these adverse effects to occur from tuberculous involvement. Then, too, not only the acute inflammatory process but even the healing phase may by cicatrization produce undue sequelae. The tracheobronchial passages, the mediastinal vascular structures, the esophagus, the heart, and pericardium may separately or in combination be involved by the mediastinal tuberculous process. There are, moreover, mediastinal tuberculous lesions which are of significant size to be recognized on chest roentgenograms or at thoracotomy, but which are asymptomatic at least at the time of their first recognition.

The effects on these various mediastinal structures may be produced by extrinsic, erosive, or intrinsic involvement. The effects on the tracheobronchial tree may lead to various forms of pulmonary suppurative disease and/or atelectasis. Thus, the attention of the physician may be directed to the secondary effect at the risk of overlooking the primary cause, mediastinal tuberculosis.

Diagnostic procedures of each type of involvement are sometimes quite extensive before the nature of the process is understood. These include bronchoscopy, special roentgenographic techniques, angiocardiography, and even exploratory thoracotomy.

The treatment of tuberculous disease of the mediastinum is concerned with treatment of the tuberculous infection (the primary problem) and with other treatment directed to the secondary effects whether these are bronchial, vascular, or esophageal in nature. Specific antituberculous measures and, in addition, surgical measures are combined. Additional forms of therapy may be needed for correction of other forms of inflammatory and obstructive phenomena.

SUMMARY

Mediastinal tuberculosis is a fairly frequent, though often unrecognized, condition. Its secondary clinical manifestations frequently obscure the primary cause

and often direct the clinician's thoughts to some other condition. Mediastinal tuberculosis, either through its active granulomatous inflammatory reaction or by its healing proliferative process, may involve (*1*) the tracheobronchial air passages, (*2*) the esophagus, or (*3*) the mediastinal vascular structure. The involvement may produce numerous types of parenchymal pulmonary infection, superior vena caval obstruction, or esophageal dysphagia. The treatment resolves itself into two phases: first, therapeutic endeavors directed at the primary problem of the tuberculous infection; and, second, therapy designed toward the relief of symptoms arising secondarily as a result of bronchial, vascular, or esophageal obstruction. The treatment is, therefore, both medical and surgical.

SUMARIO

El Problema de la Tuberculosis del Mediastino

La tuberculosis mediastínica es una afección bastante frecuente, aunque a menudo no se reconoce. Sus manifestaciones clínicas secundarias eclipsan frecuentemente la causa primaria y a menudo desvían la mente del clínico hacia alguna otra dolencia. La tuberculosis del mediastino, ya por su reacción inflamatoria granulomatosa activa o por su proceso proliferante curativo, puede afectar: (*1*) las vías aéreas tráqueobronquiales, (*2*) el esófago o (*3*) el tejido vascular del mediastino. La invasión puede producir numerosas formas de infección del parénquima pulmonar, oclusión de la vena cava superior o disfagia esofágica. El tratamiento se resuelve en dos fases: primera, esfuerzos terapéuticos encaminados al problema primario de la infección tuberculosa, y segunda, terapéutica destinada al alivio de los síntomas surgidos secundariamente a consecuencia de la oclusión bronquial, vascular o esofágica. El tratamiento es, por consiguiente, tanto médico como quirúrgico.

RESUME

Le problème de la tuberculose médiastinale

La tuberculose médiastinale est assez fréquente, bien que souvent non reconnue. Ses manifestations cliniques secondaires obscurcissent fréquemment l'étiologie primitive et entraînent souvent la pensée du clinicien vers une autre cause. La tuberculose médiastinale, soit par la réaction inflammatoire granulomateuse, soit par son processus cicatriciel fibreux, peut intéresser: 1) les voies respiratoires trachéobronchiques, 2) l'oesophage, 3) les structures vasculaires médiastinales. Ces lésions peuvent entraîner de nombreuses formes d'infection du parenchyme pulmonaire, l'obstruction de la veine cave supérieure ou la dysphagie oesophagienne. Le traitement comporte deux phases: premièrement, la thérapeutique dirigée vers le problème initial qui est l'infection tuberculeuse; deuxièmement, le traitement destiné à remédier aux symptômes secondaires résultant de l'obstruction bronchique, vasculaire ou oesophagienne. En conséquence le traitement est à la fois médical et chirurgical.

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PLATES I–VI

PLATE I

Case 8. An 18-year old airman was admitted to the hospital because of fever, cough, and malaise. He was treated at a dispensary for one week prior to his admission with 600,000 units of intramuscular penicillin and 8 gm. of sulfasoxazole daily. He failed to respond to this therapy and continued to have a daily temperature to 102° or 103° F. On admission, dullness, decreased expansion, and bronchial breathing of decreased intensity over the right upper lobe were noted.

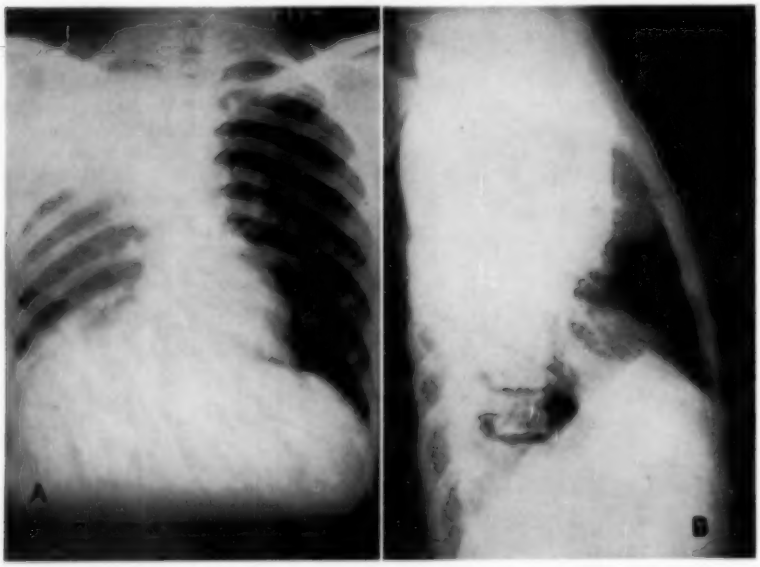
The leukocyte count was 16,700 per cu. mm. with polymorphonuclears, 74 per cent; lymphocytes, 8 per cent; eosinophils, 7 per cent. Urinalysis was negative. Blood cultures and bronchial aspirate cultures were negative for pathogenic bacteria, including *Mycobacterium tuberculosis*. The skin reactions were negative for histoplasmin, coccidioidin, and first-strength PPD, but the reaction to PPD became positive before discharge from the hospital.

The chest roentgenogram revealed a nonair-containing right upper lobe with some elevation and tenting of the right diaphragm (*A* and *B*).

Bronchoscopy was performed, and the right upper lobe bronchus appeared thickened and compressed by extrinsic pressure. A small amount of secretion was aspirated which produced no pathogenic organisms on culture.

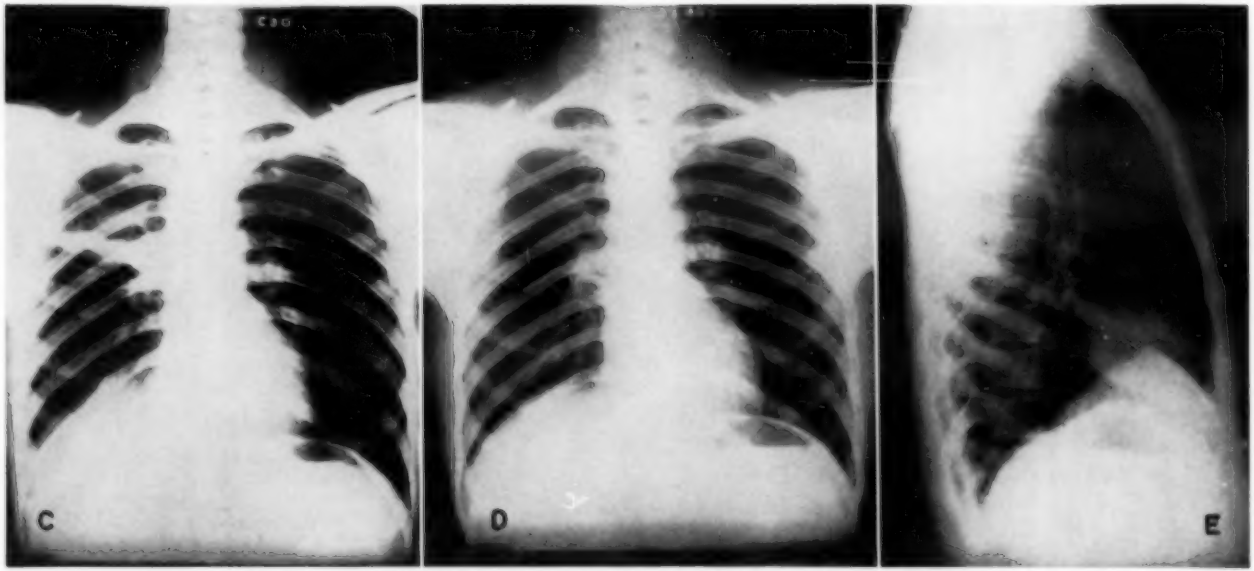
Symptomatic therapy resulted in clinical improvement and a gradual clearing of the upper lobe density with a residual of tenting of the right diaphragm (*C*, *D*, and *E*).

This represents a lobar atelectasis due to extrinsic pressure from enlarged primary tuberculous infected lymph nodes, a so-called epituberculosis.



A. A postero-anterior chest roentgenogram showing the dense atelectatic appearing area of the right upper lobe.

B. The right lateral roentgenogram also taken on admission showing the same involvement as noted in the postero-anterior view. The tenting of the diaphragm is easily seen.



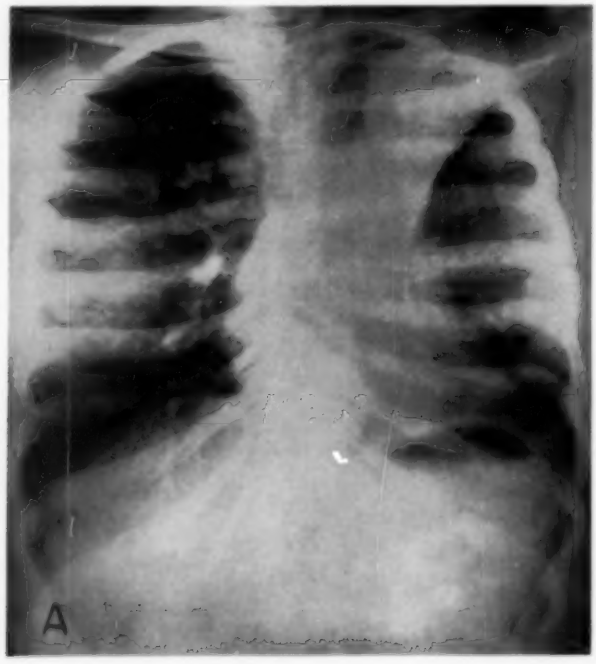
C. A postero-anterior view taken thirty days later showing the beginning of clearing of the right upper lobe while the patient was on symptomatic therapy.

D. A postero-anterior view taken sixty days after admission, showing complete clearing of the right upper lobe. The purified protein derivative skin reaction became positive one week before this roentgenogram was taken.

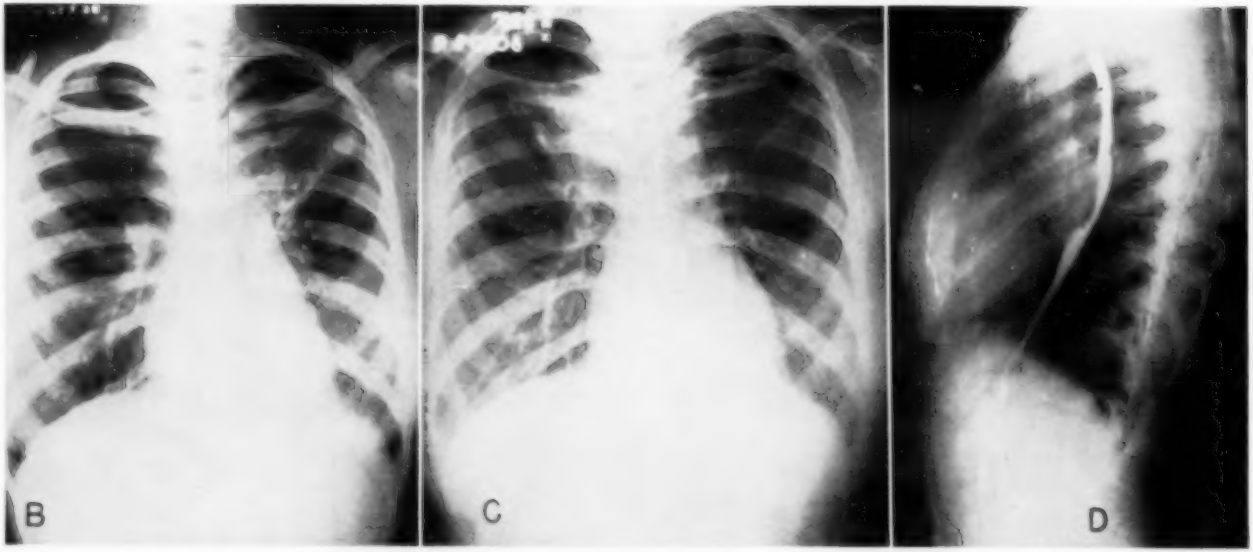
E. Film taken on the same date as *D*, the right lateral view showing the right upper lobe to be now clear. The tenting of the diaphragm by adhesions is again noted.

PLATE II

Case 6. A 20-year-old Negro female entered the hospital because of fever and general malaise. The tuberculin skin reaction was positive. Bronchoscopy was not performed. It was thought that the patient's disease represented mediastinal tuberculosis, and after streptomycin-PAS therapy clearing of the left upper lobe was noted after three months. It was also noted, however, that a mediastinal mass now presented itself on the right side (*B*). Even under therapy this became larger while the left upper lobe completely cleared. When the patient swallowed barium (*D*) at this time, the large mediastinal nodes pressing on the esophagus could be seen. This patient is still undergoing treatment and the masses are becoming smaller.



A. A postero-anterior roentgenogram of the chest showing the segmental atelectasis of the left upper lobe on admission.



B. Same view as *A*, taken three months later, showing the clearing of the left upper lobe atelectasis but the development of a mediastinal mass on the right in the superior mediastinum.

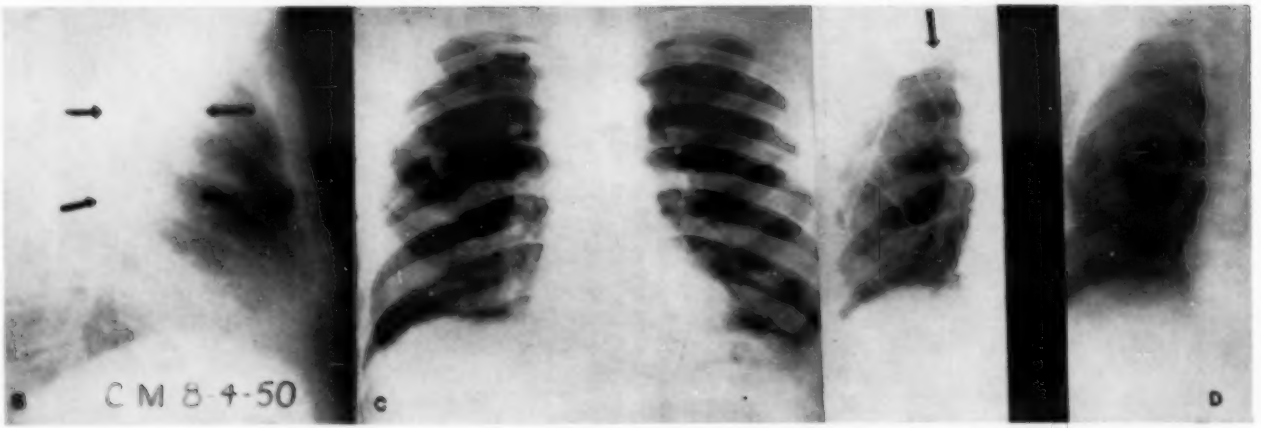
C. The postero-anterior view of the chest now shows complete clearing of the left upper lobe, but the right sided mediastinal nodes have become much larger.

D. A lateral roentgenogram of the chest demonstrating the enlargement of the mediastinal nodes displacing the esophagus backward.

PLATE III

Case 7. A 30-year-old Negro male entered the hospital with pain in the right chest and arm and exertional dyspnea of one month's duration. Postero-anterior chest film showed a lobulated mass in the right paratracheal region (*A*). Lateral view (*B*) showed the mass to be central in location. Bronchoscopy revealed extrinsic compression of trachea and right main stem bronchus. Angiocardiography showed complete obstruction of the subclavian vein at the right innominate vein (*D*). The tuberculin skin reaction was strongly positive. Thoracotomy revealed a densely adherent paratracheal lymph node mass, which showed tuberculosis of lymph nodes histologically on biopsy of part of the mass. A prolonged course of streptomycin postoperatively has been followed by regression of the node mass (*C*) and complete relief of subclavian vein obstruction (*D*—right).

A. Postero-anterior view showing the mediastinal enlargement outlined by arrows.



B. The right lateral roentgenogram showing the mass outlined by arrows.

C. Considerable reduction may be noted in the size and extent of the mass after partial surgical therapy and three months of antimicrobial therapy.

D. Angiogram taken before operation and therapy (left) showing complete obstruction to the superior vena cava with extensive collateral circulation. On the right the appearance of the angiogram of the superior vena cava shows a normal shadow with filling of the right atrium.

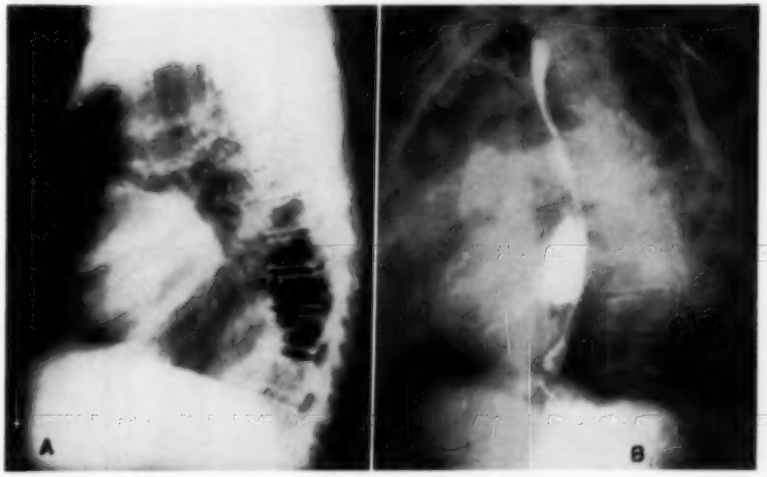
PLATE IV

Case 8. H. L., a 60-year-old white landscaper, was admitted to the hospital on September 6, 1951, with a chief complaint of shortness of breath for three months. Anorexia and a dull ache of the lower back were also present. Five months before admission he began to have a productive cough with yellowish sputum. Substernal burning was noted and became worse after solid food. Fifty pounds were lost during the two months before admission. Much of this weight loss was attributed to a liquid diet. Hemoptysis, nausea, vomiting, or melena were never noted. Chest roentgenograms before admission disclosed areas of pulmonary infiltration. He was then admitted to the hospital. The past history and family history were noncontributory.

The physical examination showed a chronically ill male. The temperature was 99.8° F.; the pulse was 100 per minute; and the respirations, 28 per minute. The blood pressure was normal. Decreased expansion of the left side of the chest with dullness were found, diminished to absent breath sounds and many rales over the upper portions of the left chest. The heart was normal except for a slight shift to the right and an occasional premature ventricular contraction. The fingers were clubbed. The rest of the physical examination was normal.

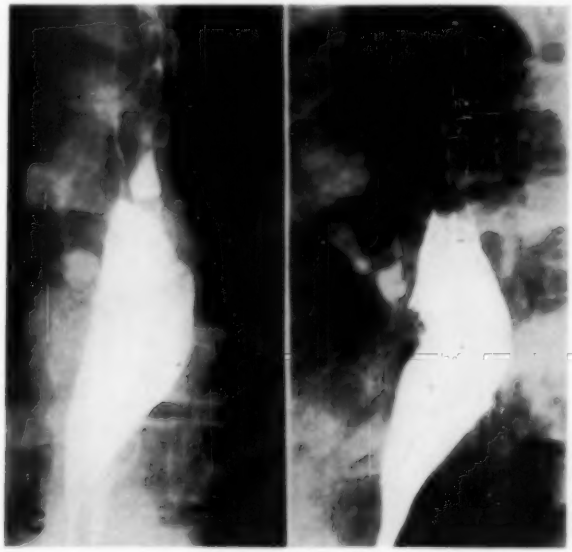
Study showed that the patient had a left-sided tuberculous pleural effusion. The sputum was positive for tubercle bacilli. A barium study of the esophagus revealed a large traction type of diverticulum of the midportion of the esophagus.

PLATE IV



A. A lateral view of the chest showing the pulmonary infiltration involving the upper and lower lobes.

B. An oblique roentgenogram of the chest with barium swallow, showing the large esophageal diverticulum located in the mid esophagus.



C. A spot film view of the large diverticulum of the esophagus.

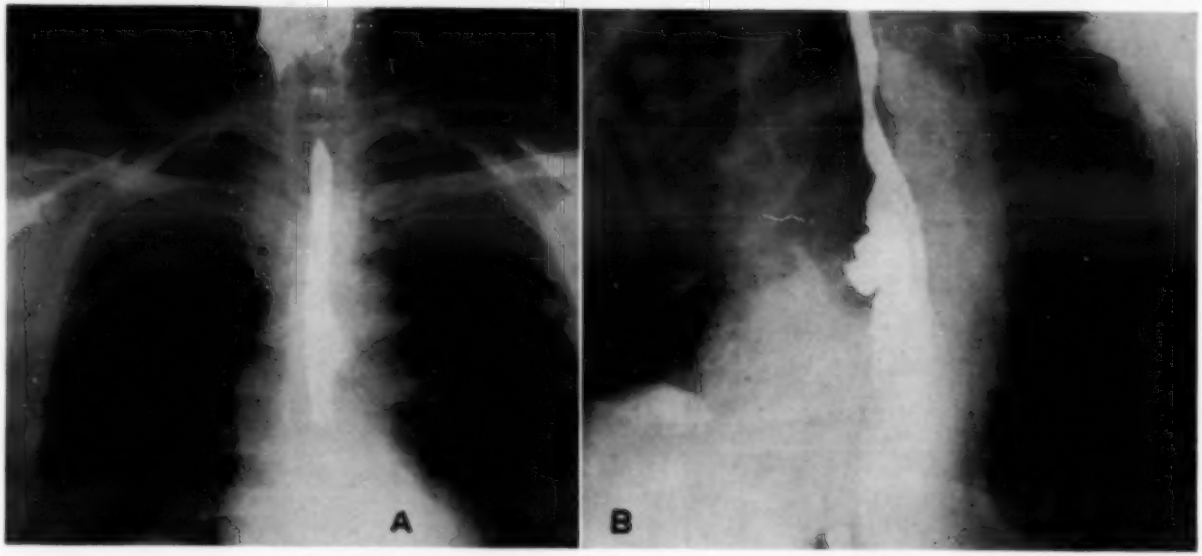


PLATE V

Case 9. A 64-year-old military veteran entered the hospital for repair and treatment of bilateral varicose veins. These had been a complaint for thirty years but, because of a large chronic ulcer of the left medial malleolar area, surgery was recommended. There were no other symptoms referable to any system. The chest was normal on physical examination.

The admission photofluorogram of the chest had a questionable right basilar pulmonary mass. On further studies made with barium swallow, a large traction diverticulum was noted in the midportion of the esophagus and some evidence of encroachment by other nodes. A tuberculin skin test, which was done later, was found to be definitely positive to first-strength PPD.

The patient denied any symptoms referable to his gastrointestinal tract. After ligation and stripping of his veins bilaterally, he was discharged. Fluoroscopic and further roentgenographic studies showed that the suspected abnormality of the right lower lobe was an anomalous diaphragmatic shadow.

A. Postero-anterior view of the chest with barium swallow showing the diverticulum located in the midportion of the esophagus and the elevated diaphragmatic shadow on the right side.

B. A lateral view showing the extensive diverticulum in the midportion of the esophagus.

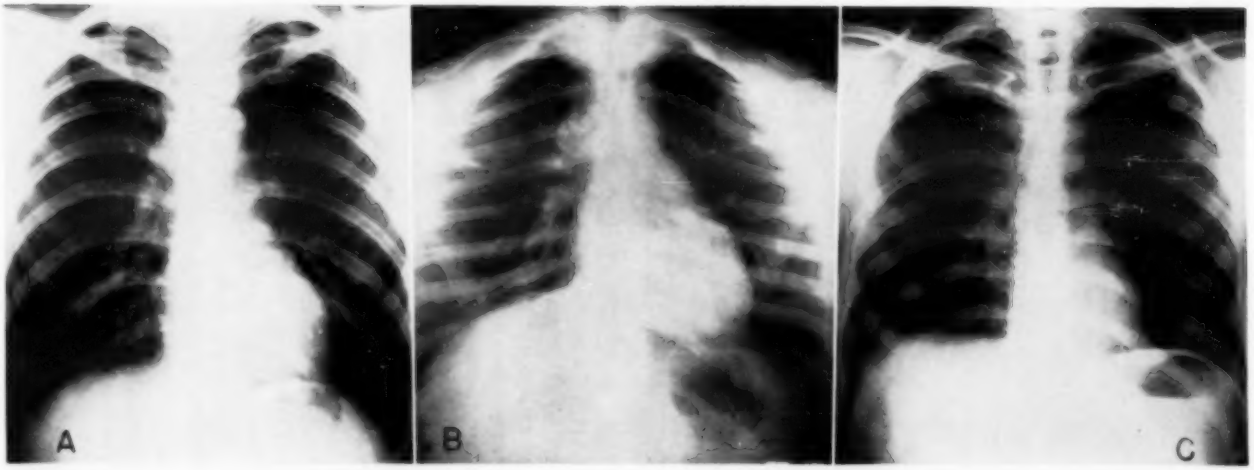


PLATE VI

Case 10. An 18-year-old male was admitted to the hospital because of a dry cough and chilly sensations. There was nothing significant in either his past or family history. A routine chest roentgenogram disclosed a right-sided paratracheal mass. The physical examination was negative and all laboratory studies were within normal limits. The tuberculin skin reaction was positive with first-strength PPD.

With symptomatic therapy, his tracheobronchial symptoms disappeared after four days. Further investigation of the mass, including bronchoscopy, angiocardiography, and gastric lavage cultures, were all normal or negative.

A right thoracotomy was performed, and a tuberculoma was removed in the notch of the superior vena cava and azygos vein. After a period of three months of bed rest and antimicrobial therapy, he was discharged.

A. Postero-anterior view of the chest showing the right paratracheal mass.

B. An apical lordotic view showing the mass and the normal upper lung fields.

C. Appearance of the chest one month after removal of the mass.

LUNG BIOPSY[1,2]

DONALD B. EFFLER, HOWARD S. VAN ORDSTRAND, LAWRENCE J. McCORMACK, AND HECTOR A. GANCEDO[3]

(Received for publication November 16, 1954)

INTRODUCTION

By tradition, the patient with bilateral diffuse pulmonary disease is considered a diagnostic problem to be managed by the internist. All too frequently the diagnosis rests on inferences drawn from roentgenographic interpretations and similar indirect methods. In the past, a physician content only with specific diagnosis had to keep vigil until the post-mortem examination, if such were to be obtained. It long has been apparent that precise study of diffuse diseases of the lung is mandatory and that direct analysis of the diseased tissue is the ideal basis for diagnosis.

Needle biopsy of lung tissue has been used for years, but never has gained wide acceptance. In addition to the obvious dangers of blind needling and its attendant complications, the method is not precise. Moreover, implantation of disease in the chest wall by infection or tumor has followed in the wake of the diagnostic needle. Needle biopsy merits little support and offers hazards to condemn its use.

Scalene node biopsy enjoys widespread popularity and is a reliable method of diagnosis in selected cases. However, biopsy in the scalene area can reveal only the disease which will reflect itself in the scalene lymph nodes. A significant number of diffuse pulmonary diseases do not reveal affinity for the scalene or any other lymph node. A surgical procedure like scalene node biopsy which offers less than 50 per cent yield in diagnosis does not approach the ideal in efficiency.

Whereas the majority of qualified specialists in pulmonary disease may prefer the more conservative operation of scalene node biopsy, the present writers' enthusiasm for this procedure has waned in favor of lung biopsy.

The writers have employed a technique of lung biopsy for a period of five years and have been favorably impressed by the results. The procedure has permitted study of lung disease by histopathologic, chemical, and bacteriologic analyses. Although the method is by no means an innocuous procedure, it is considerably less radical than the conventional exploratory thoracotomy (figure 1) and may be employed in seriously ill patients who are handicapped by a low pulmonary reserve. Although this operation offers biopsy as its sole mission, it is a definitive procedure and the diagnostic knowledge gained may offer the key to therapy and possible cure.

[1] From the Departments of Thoracic Surgery, Pulmonary Diseases, and Pathology of The Cleveland Clinic Foundation; and The Frank E. Bunts Educational Institute, Cleveland, Ohio.

[2] Presented at the Interim Scientific Session of the American Trudeau Society, held at New York City, November 17, 1954.

[3] Fellow in the Department of Thoracic Surgery.

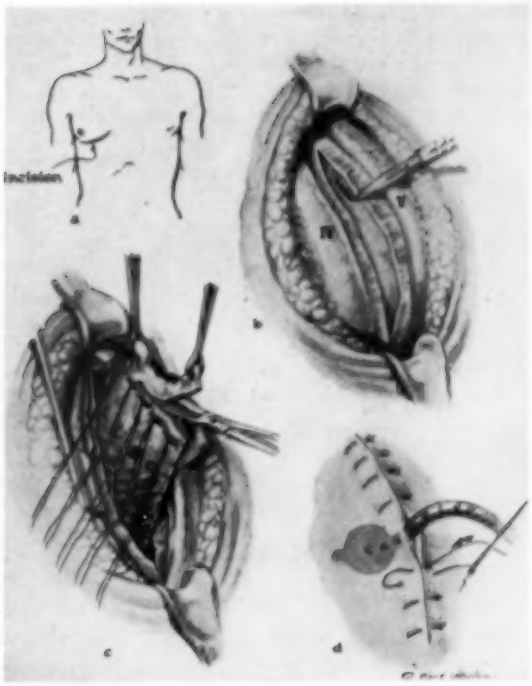


FIG. 1. Diagram of the steps involved in surgical lung biopsy. The patient in a supine position is operated upon through the anterior chest wall in this case. The intercostal incision permits visual inspection of the lung and hilum. Usually the lung can be drawn through the incision and the biopsy obtained after placing a row of hemostatic mattress sutures. A catheter is left in the thorax for water seal suction drainage.

METHOD

Anesthesia: The patient is given an appropriate preoperative sedation; a barbiturate routinely is employed. The pharynx and pyriform sinuses are treated by topical cocaine (4 per cent) with the same technique that is used for bronchoscopic examination. An endotracheal tube is inserted between the cords, and positive pressure system is applied; oxygen and nitrous oxide (50-50 combination) routinely are employed. Supplementary anesthesia is obtained by intravenous Pentothal® sodium in very small doses and a procaine (1 per cent) chest-wall block over the operative site. Emphasis is placed on employment of the lightest possible level of anesthesia.

Operation: The site of incision is plotted according to the distribution of disease as it appears on the roentgenogram. Usually an anterior approach is employed, with the patient in the supine position. After the skin has been prepared, the area is blocked with procaine (1 per cent) injected intercostally and in line with the proposed incision. The most common approach is through the anterior chest wall on either side via the fourth or fifth interspace. It is not necessary to remove either rib or cartilage. A small rib-spreader is employed for limited retraction, and the pleural

space is inspected. The lung may be retracted into the wound, assisted by positive-pressure anesthesia, and a representative area of the disease is selected. The biopsy is obtained after placing a row of hemostatic sutures around the elected site. Under sterile conditions the resected specimen may be divided into two parts and placed in glass tubes for study by the pathologists and bacteriologists. An additional speci

FIG. 2. Disposition of tissue. The histopathologic examination of the fixed tissue specimen is of greatest importance. Occasionally a specific organism may be identified by careful culture techniques, employing a small amount of the biopsy specimen. Chemical analysis of a representative section of diseased tissue is performed in selected cases, particularly those of medico-legal importance when an occupational disease is suspected.

men may be removed if chemical analysis is desired. (This was not done routinely in the present study, but is performed in selected cases.) Care is taken to secure all air leaks and bleeding points in the lung bed. The lung is re-expanded, and the incision is closed in layers with a simple form of catheter drainage.

Disposition of tissue: In the present study, specimens were processed routinely. In the majority of cases the information obtained in this manner sufficed to establish the diagnosis. However, in those patients who were suspected of having an occupational disease,

TABLE 1

DIAGNOSTIC CLASSIFICATION OF 65 CASES OF PULMONARY DISEASE, BASED ON HISTOPATHOLOGIC FEATURES OF BIOPSY SPECIMENS OF LUNGS

Categories		Number of Cases
Group 1. Granulomatous lesions		24
Berylliosis	6	
Silicosis	1	
Tuberculosis	5	
Histoplasmosis	2	
Sarcoidosis	10	
Group 2. Neoplasms		10
Primary benign	1	
Primary malignant	1	
Secondary malignant	8	
Group 3. Interstitial pneumonitis with or without fibrosis		19
Asbestosis	1	
Eosinophiliosis	1	
Fibrosing interstitial pneumonitis	17	
Group 4. Miscellaneous		12
Associated with hypersensitivity syndrome	2	
Associated with fibrocystic disease	2	
Nonspecific	8	
Total		65

e.g., berylliosis, a specimen was set aside for chemical analysis. This third method of analysis may require a fairly generous biopsy of diseased lung; ideally, a specimen weighing more than 5 gm. should be taken and kept in a chemically clean glass container.[4]

RESULTS

Lung biopsy has been performed in 65 patients at the Cleveland Clinic. There was one death; no significant morbidity has been attributed directly to the procedure.[5] This is of particular significance because the procedure has been employed in patients who suffered serious impairment of pulmonary function; many of those patients had orthopnea, pulmonary arthropathy, and visible cyanosis.

It would serve little purpose to list diagnoses in all 65 cases. Instead, an effort has been made to group these cases into four categories based on the histopathologic features of the biopsy specimen.

The four categories are: *granulomatous lesions* (24 cases), *neoplasms* (10 cases), *interstitial pneumonitis with or without fibrosis* (19 cases), and *miscellaneous* conditions (12 cases) (table 1).

[4] Chemical analyses of the biopsy specimens in this series were performed in the Kettering Laboratories, University of Cincinnati, Cincinnati, Ohio.

[5] One patient died in the hospital. He was a 48-year-old man with severe pulmonary asbestosis who suffered a massive myocardial infarction on the fifth postoperative day and died. This case is considered a surgical mortality, but indirectly related to the procedure.

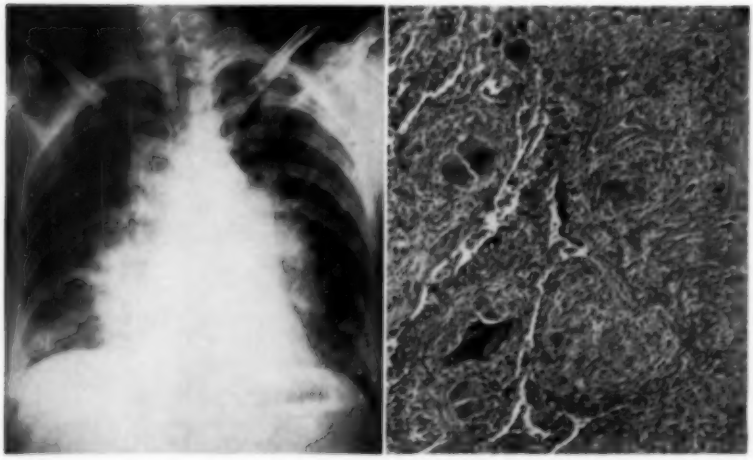


FIG. 3. Roentgenogram of a 43-year old white female who presented a history of progressive exertional dyspnea of approximately six months' duration. The lung biopsy revealed the typical gross and microscopic appearance of sarcoidosis. The biopsy was performed in January, 1951. The Kveim test was not available at that time.

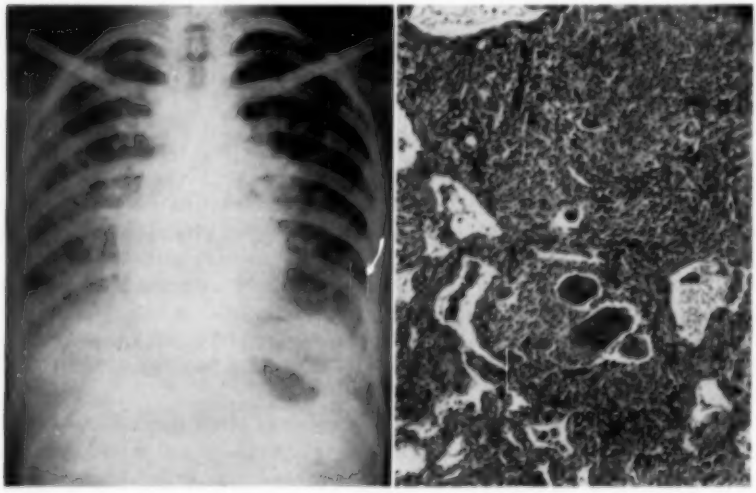


FIG. 4. Roentgenogram of a 21-year old white female who had been employed for several years by a fluorescent lamp manufacturer. At the time of lung biopsy this patient required continual oxygen therapy. The findings by histopathologic examination were compatible with berylliosis; chemical ash studies confirmed the suspicion of beryllosis. This patient has been maintained for a period of almost four years on steroid therapy.

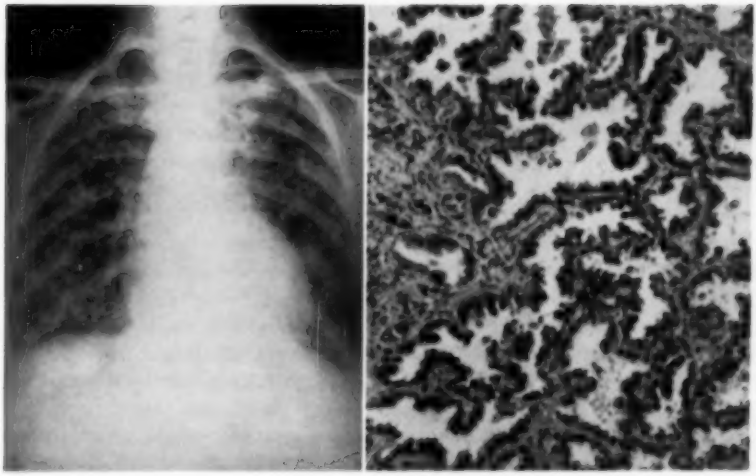


FIG. 5. Roentgenogram of a 50-year-old white female who presented no symptoms of pulmonary disease. Lung biopsy demonstrated a diffuse, multicentric type of papillary adenocarcinoma, primary in the lungs. This tumor is nonsecretory in type. Death occurred approximately one year after biopsy.

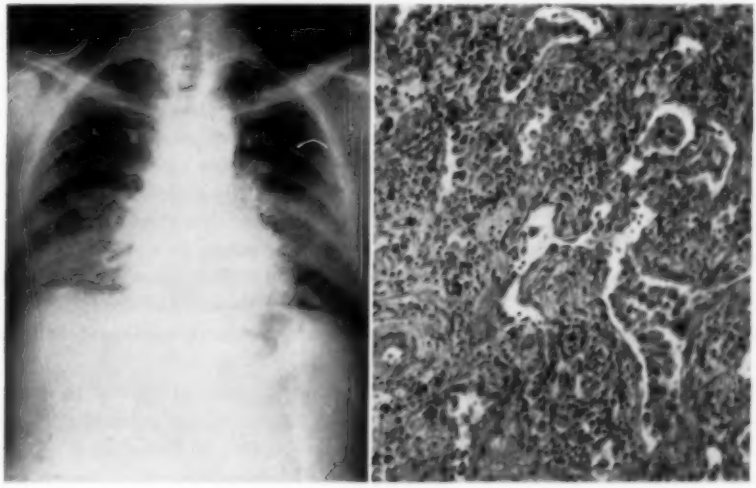


FIG. 6. Roentgenogram of one of 17 patients in this series with interstitial pneumonitis (Hamman-Rich Syndrome).

DISCUSSION

Surgical lung biopsy is a direct approach to diagnosis of diffuse pulmonary disease. Although its merits are obvious, the procedure is not to be used as a routine procedure in all such cases. More frequently than not, the clinical diagnosis may be established by less radical measures of physical diagnosis: skin tests, bacteriologic study, and cytologic examination. Only when these usual measures have been applied and have failed to provide a diagnosis should lung biopsy be performed.

With better understanding of the disease processes, the eventual need for lung biopsy will be lessened. As examples, berylliosis and sarcoidosis may be considered. These represent two of the entities listed under the heading of granulomatous diseases. In suspected cases of sarcoidosis, application of the Kveim test has drastically reduced the number of patients who might have been candidates for lung biopsy. It is the present policy to defer biopsy for a period of six weeks whenever the Kveim test is employed; if the Kveim test is positive, the diagnosis is accepted without confirmation by lung biopsy. Although there are 10 cases of sarcoidosis in the present series, only one has been added in the past two years. This experience has been paralleled in those patients with pulmonary berylliosis; although 6 patients were subjected to lung biopsy for confirmation of diagnosis, there have been no additional cases in the past eighteen months. Application of sensitivity studies by patch test (Curtis and Van Ordstrand) in those patients whose clinical histories make the diagnosis suspect has reduced the need for biopsy.

At present, the indications for lung biopsy are twofold: in diffuse pulmonary disease, in which all reasonable studies have been exhausted and the diagnosis has not been established; and in selected cases in which the possible medicolegal, occupational disease aspect may be of importance. There are advantages to lung biopsy which may be considered as indications; these are obvious when selection of therapeutic agents and determination of prognosis are considered in diffuse pulmonary disease. The wide variety of pathologic processes in the comparatively small group of 65 patients in the present study makes it apparent that therapy without diagnosis would be both inefficient and dangerous. For example, berylliosis may yield a gratifying symptomatic response to steroid therapy, a treatment that might be strongly contraindicated in certain cases of tuberculosis. Furthermore, one case of eosinophiliosis appeared to respond dramatically to arsenical therapy, an agent which certainly would not have been used if the undiagnosed case had been treated empirically.

It is difficult to assess the value of a procedure by its contribution to medical knowledge; yet it is obvious that a specific diagnosis of diffuse pulmonary disease in the living patient must lead to better understanding of that disease. Experience with 17 patients in whom the diagnosis of fibrosing interstitial pneumonitis was made by lung biopsy has been encouraging and will stimulate further study in this direction.

SUMMARY

Lung biopsy without exploratory thoracotomy has been employed as a diagnostic aid in 65 patients with diffuse pulmonary disease. A description of the method as applied at the Cleveland Clinic is presented. It is believed that the procedure of lung biopsy has a definite but limited usage.

SUMARIO

Biopsia Pulmonar

En 65 enfermos que tenían afección pulmonar difusa, se empleó la biopsia del pulmón como auxiliar diagnóstico. Preséntase una reseña del método tal como se aplica en la Clínica de Cléveland. El procedimiento de la biopsia pulmonar tiene aplicación bien definida pero limitada, discútense sus méritos manifiestos.

RESUME

Biopsie pulmonaire

La biopsie pulmonaire a été utilisée comme procédé de diagnostic chez 65 malades atteints de lésions pulmonaires diffuses. La technique, telle qu'elle est appliquée à la Cleveland Clinic, est décrite. L'emploi de la biopsie pulmonaire est bien établi, mais limité; ses avantages indiscutables sont commentés.

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UNILATERAL AND BILATERAL FUNCTIONAL CHANGES ATTENDING PHRENIC CRUSH WITH AND WITHOUT CONCOMITANT PNEUMOPERITONEUM[1, 2]

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With the technical assistance of Betty L. Vestal

(Received for publication August 18, 1954)

Introduction

It was the purpose of a recent report (1) to describe the change in midposition of the lungs produced by artificial pneumoperitoneum in young men with pulmonary tuberculosis. The present work is an extension of similar observations to another form of collapse therapy, phrenic crush.

In both of these studies, reductions in the functional residual capacity (pulmonary midposition) were used as indices of collapse. Since phrenic crush is a unilateral procedure, the measurement of this volume for each lung individually would permit more specific detection of changes than would the usual determinations of the functional residual capacity of both lungs. This unilateral procedure was therefore applied in some of the patients. The availability of this difficult technique, the scarcity of published observations regarding the functional consequences of phrenic nerve interruption, and the potential hazard this operation presents to the candidate for resectional therapy were among the considerations which prompted the interest of the present writers.

Materials and Methods

The patients were 16 young men with unilateral tuberculosis admitted from active duty to the tuberculosis service of the United States Naval Hospital, St. Albans. Each patient was studied before as well as after a phrenic crush, performed either as the sole procedure (10 patients, table 1) or in combination with artificial pneumoperitoneum (7 patients, table 2). One of these patients (No. 0732) was studied both after phrenic crush and again after pneumoperitoneum had been added to his treatment. Combined spirometry and a modification of the Darling open circuit method (2) were used in measuring the subdivision of the total capacity of *both* lungs. Four of the 10 patients treated with phrenic crush *only* and 6 of the 7 patients given both phrenic crush *and* pneumoperitoneum also had pre- and post-treatment *unilateral* total lung capacity determinations (Darling). In an additional 5 patients, these unilateral measurements were carried out postoperatively only.

Unilateral functional residual capacities were measured for each individual lung simultaneously after the patient had performed the usual bronchospirometric routine (Carlens) (3) while breathing room air. The period of oxygen breathing during the Darling procedure was increased to nine minutes rather than the usual seven, this increment of time being required to reduce the concentration of nitrogen in "alveolar" air to reasonable levels.

[1] From the Cardiopulmonary Function Laboratory, United States Naval Hospital, St. Albans, New York.

[2] This work was aided by a contract between the Office of Naval Research, Department of the Navy, and the State University of New York, NR 105 051.

TABLE 1

PRE- AND POSTOPERATIVE UNILATERAL AND BILATERAL FUNCTIONAL DATA IN MEN TREATED WITH PHRENIC CRUSH*

Patient Number	Age	Before or After Crush Right or Left	Days Before or After Crush	Functional Residual Capacity			Residual Volume			Vital Capacity			Total Lung Capacity			Ventilation Right Per Cent	Oxygen Consumption Right Per Cent	Maximum Breathing Capacity	
				Right Per Cent	Sum cc.	Bilateral cc.	Right Per Cent	Sum cc.	Bilateral cc.	Right Per Cent	Sum cc.	Bilateral cc.	Right Per Cent	Sum cc.	Bilateral cc.			L. Min.	Per Cent Predicted
0644	25	Before	7	49	2,416	2,496	48	1,368	1,266	54	5,070	5,679	53	6,438	6,945	65	53		
		After, right	33	52	2,444	2,390	51	1,558	1,270	54	3,786	5,453	53	5,344	6,723	63	54		
				†				1,324			5,453			6,777					
0732	31	Before	5	41	3,029	3,040	44	1,444	1,755	44	5,065	5,095	44	6,509	6,850	31	46	179	138
				†				1,744						6,809					
		After, right	8	45	3,030	2,945	50	1,409	1,890	45	4,131	4,475	47	5,540	6,365	50	50	130	
				†				1,975						6,106					
0720	20	Before	13	60	2,000	1,765	56	1,165	920	59	3,085	5,135	58	4,250	6,055	56	64		
				†							5,135			6,300					
		After, left	10	60	2,184	2,024	52	1,162	1,280	62	3,499	4,124	60	4,661	5,404	50	42		
				†							4,124			5,285					
0746	21	Before	2	60	2,191	2,417	63	1,140	1,502	59	2,751	4,092	59	3,891	5,594	62	53	173	121
				†				1,276			4,092			5,468					
		After, left	8	66	1,908	1,797	60	1,049	940	71	2,909	2,823	68	3,958	3,763	74	69	108	
0619	21	Before	9			1,469			694	57	3,575	3,740			4,434	61	61		
		After, left	19	69	1,494	1,685	62	834	1,015	72	2,232	2,775	69	3,096	3,790	75	81		
				†							2,775			3,609					
0755	22	Before	7			2,795			1,770	52	3,200	3,777			5,547	63	60		
		After, right	11	54	2,800	2,868	55	1,916	1,851	47	2,950	3,166	50	4,896	5,017	55	49		
0809	20	Before	6			3,585			1,805	53	5,085	5,085			6,890	62	59	107	69
		After, right	11	54	2,759	3,394	59	1,502	1,688	46	4,419	4,833	49	6,011	6,521	50	55	102	
				†	3,297														
0841	20	Before	1			1,961			1,039	51	4,298	4,452			5,491	52	56		
		After, right	14	44	1,823	1,783	52	799	781	42	3,355	3,554	44	4,154	4,335	29	44		
0730	23	Before	4			2,354			1,293	58	3,039	3,632			4,925	63	72		
		After, left	9			2,434			1,519	78	1,878	2,786			4,305	84	74		
				†							2,786								
0757	21	Before	6			2,202			1,095			5,203			6,298	72	74	182	125
		After, right	9			2,161			1,101			4,019			5,120	64	60	145	

* Unilateral volumetric data include sum of the two lungs and the per cent of this amount contributed by the right lung.

† Adjusted (*see* text).

TABLE 2

PRE- AND POSTOPERATIVE UNILATERAL AND BILATERAL FUNCTIONAL DATA IN MEN TREATED WITH PHRENIC CRUSH AND PNEUMOPERITONEUM*

Patient Number	Age	Before or After Crush Right or Left	Days Before or After Crush	State of Pneumoperitoneum	Functional Residual Capacity			Residual Volume			Vital Capacity			Total Lung Capacity			Ventilation Right Per Cent	Oxygen Consumption Right Per Cent	Maximum Breathing Capacity	
					Right Per Cent	Sum cc.	Bilateral cc.	Right Per Cent	Sum cc.	Bilateral cc.	Right Per Cent	Sum cc.	Bilateral cc.	Right Per Cent	Sum cc.	Bilateral cc.			L/Min.	Per Cent Predicted
0651	35	Before	2	none	51	3,487	3,863	50	2,222	2,416	52	5,265	6,020	51	7,487	8,436	64	56		
		After, right	31	fair	43	3,109	3,466	45	1,776	2,374	42	3,925	4,267	43	5,701	6,641	56	51		
					†				2,017						5,942					
0681	22	Before	14	none	37	1,643	1,640	59	1,036	1,105	56	2,513	4,463	57	3,549	5,570	59	55	135	96
					†							4,465			5,501					
		After, right	8	poor	46	1,496	1,632	43	723	887	45	3,326	4,035	45	4,049	4,922	39	49	95	
					†							4,035			4,717					
0689	23	Before	6	none	48	2,647	2,901	45	1,347	1,876	48	3,531	4,395	47	4,878	6,271	52	45	154	116
					†				1,627			4,395			6,193					
		After, right	7	good	46	2,411	2,590	49	1,412	1,465	44	2,975	3,670	46	4,387	5,135	50	50		
					†							3,670			5,082					
		After, right	94	good	33	2,121	1,792	30	1,172	1,071	32	2,762	2,880	31	3,934	3,950	36	41	93	
					†	1,893														
0756	27	Before	7	good	48	2,743	2,901	50	1,346	1,647	50	5,145	5,140	50	6,491	6,787	70	52	107	80
					†				1,480						6,625					
		After, right	14	good	37	3,265	3,048	31	2,366	1,824	46	4,538	4,630	41	6,904	6,454	71	69	100	
					†				2,041						6,578					
0732	31	Before	5	none	41	3,029	3,040	44	1,444	1,755	44	5,065	5,095	44	6,509	6,850	31	46	179	138
					†				1,744						6,809					
		After, right	8	none	45	3,030	2,945	50	1,409	1,890	45	4,131	4,475	47	5,540	6,365	50	50	130	
									1,975						6,106					
		After, right	49	good	38	2,787	2,301	42	1,591	1,109	42	3,906	4,238	42	5,497	5,347	47	52	117	
0505	24	Before	4	none	64	2,163	2,073	62	1,081	673	59	4,501	4,725	59	5,582	5,398	65	61		
					†				763						5,264					
		After, left	28	good	82	1,359	1,696	83	714	847	72	3,430	3,817	74	4,144	4,664	80	74		
					†	1,563														
0508	24	Before	1	none			3,015			1,585	50	4,000	4,501			6,086	54	40		
		After, right	65	good	50	1,962	2,185	58	1,110	1,105	41	3,506	3,775	45	4,616	4,880	51	40		
0735	30	Before	5	good															97	79
		After, right	2	good															84	

* Unilateral volumetric data include sum of the two lungs and the per cent of this amount contributed by the right lung.

† Adjusted (see text).

Thirty-three of the forty-five "alveolar" samples which were uncontaminated during delivery contained less than 2.50 per cent nitrogen. Of the remaining twelve samples, one was in excess of 5.00 per cent (7.24 per cent).

The most serious sources of error in the technique for measuring the unilateral functional residual capacity, once air leaks have been eliminated, result from the effects of bronchospirometry on midposition and on the ability to deliver lung volumes corresponding to those obtained with a standard oral spirometric coupling. In some instances, poor checks between unilateral and bilateral figures were attributable to difficulties in delivering the expiratory reserve. On such occasions, data were included when the functional residual capacity (4) was acceptable (within 15 per cent of bilateral value) and the residual volume unacceptable (not within 15 per cent of bilateral value), provided use of the expiratory reserve obtained with oral coupling brought the resultant bronchospirometry residual volume within the acceptable range. Similarly, patients in whom the values for unilateral and bilateral residual volumes agreed but in whom the functional residual capacities failed to correspond were included, provided the discrepancy in functional residual capacity could be reduced to 15 per cent or less by using the expiratory reserve obtained with the oral technique. In such instances, the midposition was assumed to have been misjudged at the time oxygen breathing was begun (patients Nos. 0809, 0689, and 0505). Finally, when inspiratory capacities by oral and bronchospirometric methods were discrepant in patients with otherwise technically satisfactory tracings, the oral value was used in the calculation of the total lung capacity. Measurements of the functional residual capacity, residual volume, vital capacity, and total lung capacity computed according to these principles are referred to in tables 1 and 2 as "adjusted." In each instance, the "adjusted" volume was distributed between the two lungs as had been the volume actually measured.

Twenty-one of the twenty-five comparisons between unilateral and bilateral values for the functional residual capacity which are included in this study showed a difference of less than 15 per cent[1] (exceptions: patients Nos. 0809, 0689, 0732, and 0505 with discrepancies of 19, 18, 21, and 20 per cent, respectively). Use of "adjusted" values did not, therefore, figure prominently in a primary objective of this work, i.e., evaluating the effect of phrenic crush on the unilateral midposition. The "adjusted" value was applicable in 3 of the 4 exceptions enumerated above. In the case of the fourth, patient number 0732 (third study), data were included in spite of a 21 per cent difference between unilateral and bilateral functional residual capacities and a 43 per cent difference in residual volumes. This was done because the distribution of the volumes measured bronchospirometrically corresponded to what one would anticipate both from roentgenograms and from the studies of other patients.

The measurements of bilateral functional residual capacity were performed in duplicate. Only studies in which the two determinations agreed within 10 per cent or 200 cc. were included. The mean of the two values was used in the tables. All bilateral and unilateral lung volumes were converted to body temperature, ambient pressure, saturated with water vapor (BTPS).

The maximum breathing capacity was performed in triplicate through a Collins low resistance valve by collecting a fifteen-second sample of expired air in a Douglas bag. The best of the three trials was expressed in liters per minute BTPS.

All patients had paradoxical motion of the affected hemidiaphragm at the time of the postphrenic study.

Results

Lung Volumes

Functional Residual Capacity

Phrenic crush only (table 1): Nine of the 10 patients treated with phrenic crush only failed to show a fall in the bilateral functional residual capacity ex-

[1] Per cent differences between bilateral and unilateral techniques were computed by dividing the difference between the values by the bilateral volume.

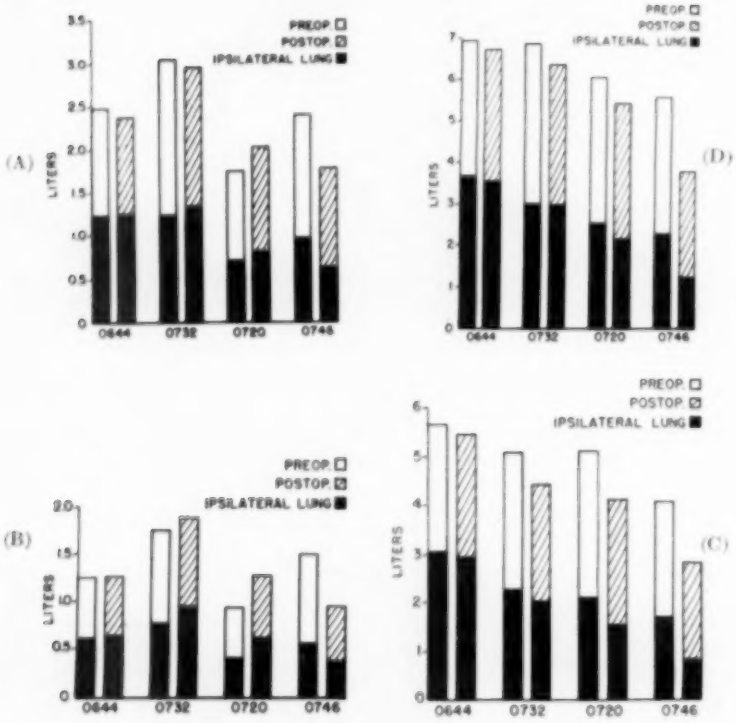

FIG. 1. Pre- and postoperative unilateral and bilateral lung volumes in 4 patients treated with phrenic crush only (table 1). *A*—functional residual capacity; *B*—residual volume; *C*—vital capacity; *D*—total lung capacity. The bilateral and ipsilateral functional residual capacity (*A*) and the residual volume (*B*) were reduced by operation in only one patient (No. 0746). Note the considerable fall in vital capacity (*C*) and total lung capacity (*D*) in patients Nos. 0720 and 0746. Patient No. 0644 is the only one of the 10 patients in table 1 who did not suffer a loss of vital capacity on the affected side exceeding 10 per cent of the preoperative value.

ceeding the errors of the analytic technique (10 per cent).[a] Similarly, 3 of the 4 patients with pre- and postphrenic unilateral determinations showed no significant (larger than 10 per cent) change in the functional residual capacity on the side of operation (figure 1*A*).[b] In one patient (No. 0746) the functional residual capacity did fall (26 per cent of the bilateral value, 37 per cent on the affected side). In 2 other patients (Nos. 0619 and 0841) whose bilateral studies indicated

[a] Per cent rise or fall computed by dividing the pre- to postoperative difference by the preoperative value.

[b] Unilateral per cent rise or fall computed by distributing the bilateral volume between the two lungs according to the per cent established for the unilateral volumes and dividing the pre- to postoperative change by the preoperative value.

no decrease in consequence of the phrenic crush, there was an abnormally low proportion of the functional residual capacity on the affected side at the time of their postoperative unilateral determinations. There was definite evidence, therefore, of a change in midposition following phrenic crush in only one of these 10 patients and presumptive evidence of a similar effect on the side of the crush in 2 other instances.

Phrenic crush plus pneumoperitoneum (table 2, figures 2A, 4 and 6): Four patients (Nos. 0689, 0732, 0505, 0508) had bilateral functional residual capacity measurements both before as well as after phrenic nerve paralysis plus a well-established pneumoperitoneum. In each of these, the functional residual capacity

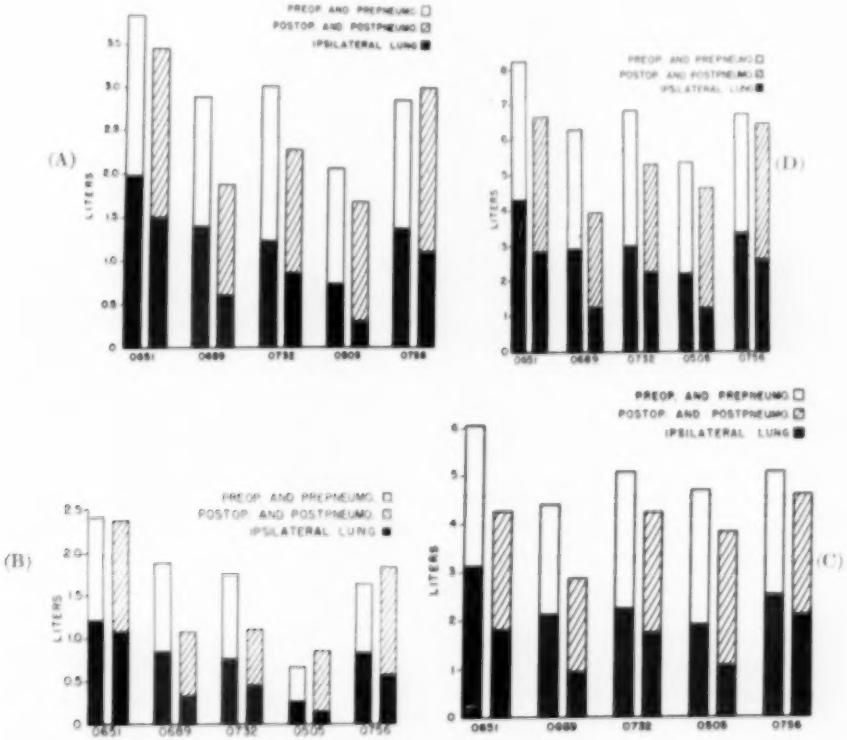

FIG. 2. Pre- and postoperative unilateral and bilateral lung volumes in 5 patients treated with both phrenic crush and artificial pneumoperitoneum (table 2). *A*—functional residual capacity; *B*—residual volume; *C*—vital capacity; *D*—total lung capacity. At the time of his preoperative study, patient No. 0756 had a well-established pneumoperitoneum. Graphs of other patients as indicated in legend. Note that there are bilateral and/or ipsilateral changes, not only in vital capacity and total lung capacity, as in figure 1, but in the functional residual capacity and residual volume as well. For an explanation of differences among these patients, see text.

decreased significantly (38, 24, 18, and 28 per cent). In addition, the first 3 of these 4 patients had pre- and post-treatment unilateral determinations. All 3 demonstrated a reduction in the proportion of the functional residual capacity contributed by the affected lung (falls of 58, 34, and 59 per cent, respectively). The fourth patient (No. 0508), who had no pre-treatment but had a post-treatment unilateral determination, was found somewhat surprisingly to have an even distribution of the functional residual capacity between the two lungs at the time of the postoperative study.

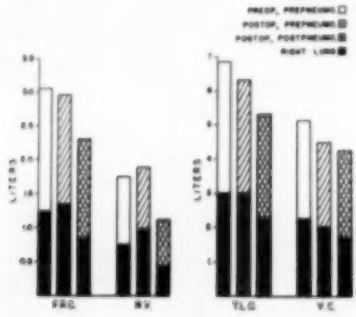

FIG. 3. Unilateral and bilateral lung volumes in patient No. 0732 studied before either phrenic crush or pneumoperitoneum, after phrenic crush, and again after pneumoperitoneum had been added (table 2). See figure 4 for roentgenograms corresponding to the three studies. Note that a fall in ipsilateral or bilateral functional residual capacity (F. R.C.) and residual volume (R.V.) was demonstrated only after pneumoperitoneum had been added.

In 2 patients (Nos. 0651 and 0681) whose pneumoperitoneum was either fair or poor at the second study and in one patient (No. 0756) with a well-established pneumoperitoneum at both preoperative and postoperative studies, comparison of the pre- and postphrenic crush bilateral functional residual capacities showed a change of only minus 10 per cent or less. All 3 patients, however, demonstrated a redistribution of the functional residual capacity so that a smaller proportion of this volume was contained in the affected lung.

The bilateral and/or unilateral functional residual capacity in each of these 7 patients, therefore, fell as a result of phrenic nerve paralysis administered in conjunction with pneumoperitoneum.

The limitations of a phrenic crush alone in collapsing the lung stood in contrast to its effectiveness when associated with pneumoperitoneum in patient No. 0732 (table 2; figures 3 and 4). This patient had shown essentially no functional residual capacity change when studied after phrenic crush alone. After pneumoperitoneum had been added to his regimen, however, there was a 34 per cent reduction in the functional residual capacity on the affected side.

The changes in midposition produced by combining phrenic nerve paralysis with pneumoperitoneum are progressive. This point is illustrated by patient

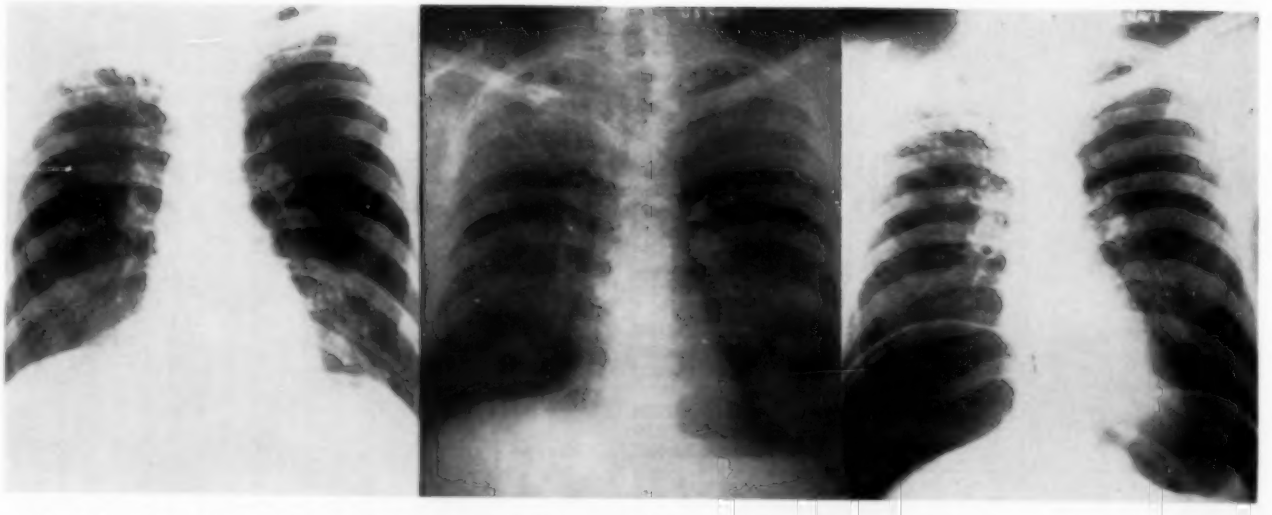

FIG. 4. Inspiratory chest roentgenograms of patient No. 0732. Reading from left to right, these films were taken before operation and pneumoperitoneum, postoperatively, and after pneumoperitoneum had been added. Corresponding volumetric studies are shown in figure 3. Note (1) equivocal effect of phrenic crush alone on level of right diaphragm and (2) elevation of both diaphragms by pneumoperitoneum with greater effect on right than on left.

No. 0689 (table 2; figures 5 and 6). This patient was studied first before any treatment, again after three weeks of pneumoperitoneum and one week following phrenic nerve paralysis, and finally once again twelve and one-half weeks later. While the functional residual capacity on the side of crush had fallen by only 14 per cent at the second study, this collapse had increased to 58 per cent at the time of the third study.

Residual Volume

Phrenic crush only (table 1): Five of the 10 patients with only phrenic crush demonstrated changes in bilateral residual volume in excess of 10 per cent. Patients Nos. 0720, 0730, and 0619 showed increases (39, 17, and 46 per cent, respectively) and patients Nos. 0746 and 0841 had decreases (37 and 25 per cent,

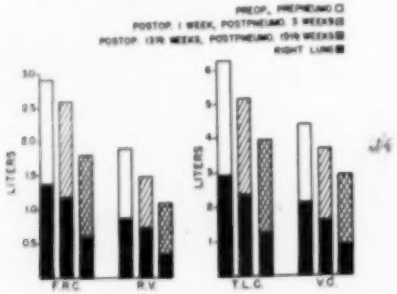

FIG. 5. Unilateral and bilateral lung volumes in patient No. 0689 studied (*a*) before phrenic crush and pneumoperitoneum, (*b*) one week after phrenic crush and three weeks after pneumoperitoneum, and (*c*) twelve and one half weeks after (*b*). See figure 6 for corresponding roentgenograms. Note: (*1*) therapy has reduced all lung volumes, (*2*) effect of time on the extent of this reduction and (*3*) selectivity of changes for the right lung.

respectively). Four patients (Nos. 0644, 0732, 0720, and 0746) had pre- and postoperative unilateral determinations (figure 1*B*). In each of these the proportion of the residual volume contributed by the affected lung was moderately increased or essentially unchanged by phrenic crush. Four patients had unilateral determinations postoperatively only (Nos. 0619, 0755, 0809, and 0841). Only one patient (No. 0619) had an unusually low per cent of this volume on the affected side.

It is evident, therefore, that the proportion of the residual volume contributed by the lung on the side of the phrenic crush was rarely decreased by this operative procedure. In addition, a fall in total residual volume in excess of 10 per cent was demonstrated in only 2 of the 10 patients.

Phrenic crush plus pneumoperitoneum (table 2; figures 2*B*, 4, and 6): Three (Nos. 0689, 0732, 0508) of the 4 patients studied prior to any treatment and subsequently, after both phrenic crush and a well-established pneumoperitoneum, were found to have decreases in bilateral residual volume exceeding 10 per cent (43, 37, and 30 per cent, respectively). The fourth patient (No. 0505) with a very

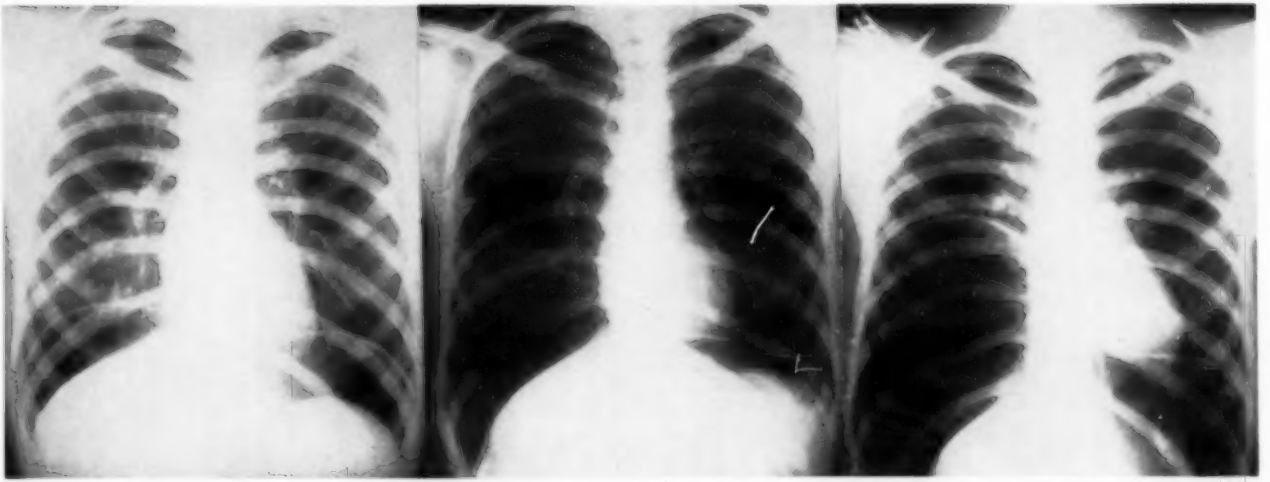

FIG. 6. Inspiratory chest roentgenograms of patient No. 0689. The film on the left was taken a few weeks before the first study, at which time the patient had not had a phrenic crush. The patient was taken off pneumoperitoneum after this roentgenogram had been taken and had no demonstrable air beneath the diaphragms at the time of his first study (figure 5). The films in the center and on the right were taken at the time of the second and third studies, respectively (figure 5). Note the changes with time and the selective effect of pneumoperitoneum on the right hemithorax in the presence of right phrenic nerve paralysis.

small residual volume had an increment of 174 cc. (26 per cent), a difference probably not representing a real change. The 2 patients (Nos. 0651 and 0681) whose pneumoperitoneum was not well established at the second study showed changes of minus 2 per cent and minus 20 per cent. Finally, patient No. 0756, who had a good pneumoperitoneum at both pre- and postoperative determinations, demonstrated an equivocal change (plus 11 per cent).

In distinction to the variability of these results relating to changes in *total* residual volume, each of the 6 patients with pre- and postphrenic *unilateral* analyses was found to have a diminished residual volume on the affected side following his operation. These data contrast with those obtained in the absence of pneumoperitoneum.

Patients Nos. 0732 (figures 3 and 4) and 0689 (figures 5 and 6) were again particularly instructive in demonstrating the consequences of pneumoperitoneum when superimposed on phrenic nerve paralysis. The former patient showed an increase of 22 per cent in the residual volume of the right lung after right phrenic paralysis had been performed. Following the addition of pneumoperitoneum, the residual volume had fallen by 40 per cent of its initial value. Similarly, although the early postpneumoperitoneum and postphrenic study of patient No. 0689 showed only a 15 per cent loss of residual volume on the right side, this decrease was augmented to 62 per cent by prolonged pneumoperitoneum.

Vital Capacity

Phrenic crush only (table 1, figure 1*C*): All patients experienced reductions in bilateral vital capacity in consequence of the phrenic crush. The extent of this fall varied from 4 per cent of the preoperative volume in patient No. 0644 to 31 per cent in patient No. 0746. The mean for the group was minus 18 per cent. Of the 9 patients with pre- and postoperative unilateral comparisons, 3 (Nos. 0644, 0732, and 0720) showed essentially no change in distribution of the vital capacity between the two lungs, while in 6 the proportion of the vital capacity contributed by the affected lung was considerably reduced. With the exception of patient No. 0644, each patient suffered a loss of the vital capacity on the affected side exceeding 10 per cent of the preoperative value (group mean = minus 31 per cent, with a range from minus 4 per cent in patient No. 0644 to minus 60 per cent in patient No. 0730).

Phrenic crush plus pneumoperitoneum (table 2; figures 2*C*, 4, and 6): Each of the 7 patients demonstrated a reduction in total vital capacity varying from 10 per cent in patients Nos. 0681 and 0756 to 34 per cent in patient No. 0689, with a group mean of 19 per cent. The per cent of the vital capacity contributed by the affected side was decreased in each instance, the mean reduction in this volume for the single lung being 34 per cent for the group, with a range from 17 per cent to 56 per cent.

With the single exception of patient No. 0644, therefore, each of the 17 patients included in this study demonstrated a significant loss of the vital capacity following phrenic nerve paralysis.

Total Lung Capacity

The changes in total lung capacity (tables 1 and 2; figures 1*D*, 2*D*, 4, and 6) paralleled those in vital capacity. The group mean fall in bilateral values was 14 per cent in patients not receiving pneumoperitoneum, with a mean unilateral loss of 17 per cent. Corresponding figures for the 7 pneumoperitoneum patients were 19 and 36 per cent, respectively. The higher figures in the latter group reflect the fact that, with 2 exceptions, the patients included demonstrated decreases not only in vital capacity but in residual volume as well.

Volumetric Ratios

Phrenic crush only: Not one of the patients treated only with phrenic nerve paralysis demonstrated a fall in either the ratio residual volume/total lung capacity or the ratio functional residual capacity/total lung capacity in consequence of the operation. Seven of the 10 patients evidenced a rise in these ratios with respect to bilateral values, and 3 of the 4 patients with pre- and postoperative unilateral lung volumes showed an increase on the operative side. No change was found in the other patients.

Phrenic crush plus pneumoperitoneum: An ipsilateral and/or a bilateral increase in these ratios was commonly observed also in the 7 pneumoperitoneum patients. Among these, however, there were 4 instances in which the value for the bilateral and/or the ipsilateral ratio either fell or remained constant.

The increase in these ratios commonly found following phrenic nerve paralysis in both patient groups reflected the disproportion between the effect of this procedure on vital capacity or inspiratory capacity and on the residual or functional residual volumes. This was particularly true of the patients treated with phrenic crush only.

VENTILATION

Maximum breathing capacity: This test was performed before and after phrenic crush in 8 patients, 5 of whom also received pneumoperitoneum. Each patient demonstrated a fall varying from 5 per cent in patient No. 0809 to 40 per cent in patient No. 0689 (group mean: 24 per cent). Three of these patients had a preoperative maximum breathing capacity below 90 per cent of predicted. The mean fall among these was 8 per cent as contrasted to 32 per cent among those with a normal preoperative maximum breathing capacity. The loss in maximum voluntary ventilatory capacity in patients with normal function was therefore pronounced and, in these 5 patients, considerably exceeded that incurred by patients in whom this value was diminished preoperatively.

Ventilation at rest: This was adversely affected on the side of operation in 11 of 16 patients, was unchanged (change of 3 per cent or less) in 3 patients, and appeared to be improved in 2 (Nos. 0732 and 0720). The paradoxical result in these latter may well have reflected the difficulties of bronchospirometry rather than the effects of operation.

OXYGEN CONSUMPTION

The proportion of total oxygen consumption taken up on the side of phrenic crush was usually affected in the same direction as the minute ventilation, increasing where the latter increased and falling where the latter fell. Accordingly, no trend could be detected with respect to the effect of phrenic crush on the ventilatory equivalent.[8] Neither was this ratio higher or lower on the side of operation than on the normal side in a significantly large proportion of the patients.

DISCUSSION

Since the volume of the lung is a measure of its distention, collapse can be detected by volumetric reductions. The primary purpose of the present study was to test the validity of employing one procedure, phrenic crush, as a means of achieving collapse. Toward this end, the midposition (functional residual capacity) of each lung and/or both lungs of the patient breathing quietly in the supine position was measured before and after phrenic crush. In the absence of pneumoperitoneum, this operation was followed by collapse in only one of 10 patients. In each of these same patients, however, this procedure inflicted a functional loss. For this group, therefore, phrenic nerve paralysis alone offered an unlikely therapeutic prospect at the cost of a certain functional loss.

These data support the observations in the limited pertinent literature. The 2 patients with unilateral volumetric studies described by Gaensler and Cugell (5) and the patient with bronchospirometric data described by Wright and associates (6) also demonstrated unilateral loss of function and, in the former study, absence of unilateral midpositional change. An unfavorable relationship between pulmonary ventilation and perfusion which resulted in poor oxygenation of arterial blood in patients with phrenic crush was described by Cournand and Richards (7). Vaccarezza and associates (8) stressed the striking deleterious effect of phrenic crush on ipsilateral oxygen uptake which they found in ten patients. There are, therefore, other data attesting adverse physiologic effects of this operation. If to these one adds the difficulties of reduced tussic force imposed by phrenic crush on a patient who may be a candidate for future resectional therapy, the case against phrenic paralysis becomes compelling indeed.

When pneumoperitoneum was added, these disadvantages were partly offset by the deflation which this supplementary procedure affords. The mean value of this collapse among 4 patients amounted to 27 per cent of the prepneumoperitoneum bilateral volume. This figure is hardly impressive, for pneumoperitoneum without phrenic crush commonly reduces the functional residual capacity 20 to 25 per cent (1, 9). However, the 3 of these 4 patients who had unilateral as well as bilateral studies demonstrated selective reductions on the affected side which were 34, 58, and 59 per cent of the prepneumoperitoneum capacity. The magnitude of these latter two striking changes is in considerable excess of

[8] Ventilatory equivalent = ventilation/oxygen uptake.

the largest one measured for both lungs in thirty-one patients with pulmonary tuberculosis (1).

Implicit in this discussion is the assumption that the primary goal of phrenic crush is deflation (collapse or "relaxation")[7] of the infected lung. Although this objective was not met without the addition of pneumoperitoneum in 9 of 10 patients, that of relative "rest" or "immobilization" of the lung was regularly achieved. Thus, the ipsilateral minute ventilation was diminished and the maximum possible changes in distention during deep breathing or coughing (vital capacity) were reduced.

The absence of a midpositional change consequent to phrenic interruption alone may be unexpected when referred to the mental image of the customary inspiratory roentgenogram, for in such a film the paralyzed hemidiaphragm is usually higher than its opposite mate. Such pictures should be correlated, not with the functional residual capacity, however, but with the total lung capacity, figures for which do show the disparity between the two sides which one would anticipate from the roentgenographic appearance. Midposition roentgenograms or fluoroscopic examinations afford a more accurate appraisal of midposition change.

In reference to the influence of lesions such as pulmonary tuberculosis on position of the diaphragm and unilateral volume, the affected lung of some patients contained less than the normal proportion of the functional residual capacity preoperatively. For instance, patient No. 0732, with a lesion on the right, had 41 per cent of the functional residual capacity on the right; and patient No. 0505, with a left-sided lesion, had 36 per cent of the functional residual capacity on the left.[8] Since the figures recorded for patients Nos. 0841 and 0619, who had unilateral studies postoperatively only, are within this range (44 per cent on the right in the former, 31 per cent on the left in the latter), these cannot in the absence of preoperative values be ascribed with certainty to the phrenic nerve paralysis on the corresponding side. Particularly is this true inasmuch as neither patient demonstrated a change in bilateral functional residual capacity in consequence of his operation. For this reason it was concluded above that only one of the 10 patients studied who had not received pneumoperitoneum demonstrated an effect of phrenic crush on functional residual capacity.

It should be noted in closing that unilateral functional residual capacity measurements in some patients provided closer estimates of changes in the single lung than could be obtained by more conventional bilateral measurements or by fluoroscopic observations. For example, the effectiveness of pneumoperi-

[7] The terms "collapse" and "relaxation" are both used in this paper in referring to decreases in the volume of air present in the lung at the midposition. The words "rest" or "immobilization" are used in describing a reduction in the phasic changes of volume associated with normal or deep breathing.

[8] In 11 patients with coin lesions or minimal pathology and with normal bronchospirometry, the functional residual capacity was found to be distributed between the two lungs in the same proportion as the vital capacity (53 per cent on the right, 47 per cent on the left).

toneum and phrenic crush in selectively changing the midposition of the affected lung in patients Nos. 0651, 0681, and 0756 would not have been ascertained by the usual technique of oral coupling.

This study suggests that, if "collapse" or "relaxation" is the objective of phrenic crush in the patient on bed rest, this procedure should be reserved for the candidate who can tolerate artificial pneumoperitoneum. The increasing effectiveness of other therapeutic approaches and the functional deterrents to temporary phrenic interruption may justify the disfavor with which this latter procedure is regarded by many for the treatment of pulmonary tuberculosis.

Summary

Sixteen young men with unilateral pulmonary tuberculosis were studied before and after being treated with phrenic crush administered either as the sole procedure or in association with artificial pneumoperitoneum. Functional analyses included pre- and postoperative measurements of the total lung capacity and its subdivisions, differential bronchospirometry, and the maximum breathing capacity. The volumetric compartments of the single lung were measured in 10 patients in an attempt to add greater precision to the detection of changes in pulmonary midposition.

The total and unilateral *functional residual capacity* fell following phrenic crush in one of the 10 patients who was given only a phrenic crush; 9 of these patients, 3 of whom had unilateral measurements, demonstrated no change in midposition of the lung in consequence of their operation. On the other hand, the functional residual capacity was reduced in each of 7 patients in whom temporary phrenic paralysis was supplemented with pneumoperitoneum. A selective effect on the side of phrenic crush was demonstrated by the 6 of these 7 patients who had unilateral measurements.

The *residual volume* was infrequently reduced by phrenic crush alone, but commonly fell in the presence of pneumoperitoneum.

Vital capacity, the unilateral contribution to *ventilation* and *oxygen uptake*, and the *maximum breathing capacity* were usually significantly reduced postoperatively.

Phrenic crush inflicted a functional loss on all patients. Only in those receiving pneumoperitoneum was a favorable effect on pulmonary midposition demonstrated.

Sumario

Alteraciones Funcionales Uni y Bilaterales que Acompañan a la Freniclasia con y sin Neumoperitoneo Concomitante

A 16 jóvenes con tuberculosis pulmonar unilateral se les estudió antes y después de tratarlos con la freniclasia aplicada ya como procedimiento único o unida al neumoperitoneo terapéutico. Los análisis funcionales comprendieron mediciones pre- y post-operatorias de la capacidad pulmonar total y sus subdivisiones, broncospirometría diferencial y la capacidad respiratoria máxima. En 10 enfermos, se midieron los compartimientos volumétricos de un solo pulmón con mira a imprimir mayor precisión al descubrimiento de alteraciones en la mesoposición pulmonar.

La *capacidad residual funcional* total y unilateral bajó después de la frenicoclasia en uno de los 10 enfermos que no recibieron más que este tratamiento; 9 de estos sujetos, en 3 de los cuales se hicieron mediciones unilaterales, no revelaron la menor alteración en la mesoposición del pulmón a consecuencia de la operación. En cambio, descendió la capacidad residual funcional en los 7 sujetos en los que se complementó con el neumoperitoneo la parálisis temporal del frénico. Mostraron efecto selectivo del lado de la frenicoclasia los 6 enfermos de estos 7 en quienes se hicieron mediciones unilaterales.

El *volumen residual* fué reducido raramente por la frenicoclasia sola, pero bajó habitualmente cuando se ejecutó el neumoperitoneo.

La *capacidad vital*, el aporte unilateral a la *ventilación* y la *toma de oxígeno* y la *capacidad respiratoria máxima* disminuyeron por lo general en forma notable postoperatoriamente.

La frenicoclasia ocasionó una pérdida funcional en todos los enfermos. Sólo en los que recibieron el neumoperitoneo se observó efecto favorable sobre la posición media.

RESUME

Modifications fonctionnelles unilatérales et bilatérales accompagnant l'écrasement du phrénique, avec ou sans association de pneumopéritoine

Seize jeunes hommes atteints de tuberculose pulmonaire unilatérale ont été étudiés avant et après avoir été soumis à l'écrasement phrénique effectué soit comme procédé unique soit en association avec le pneumopéritoine artificiel.

L'analyse fonctionnelle comprenait des déterminations postopératoires de la capacité totale du poumon et de ses subdivisions, la bronchospirométrie différentielle et la capacité de ventilation maxima. Les divisions volumétriques d'un seul poumon ont été déterminées chez 10 patients pour tenter de discerner avec une plus grande précision les variations dans la ventilation moyenne du poumon.

La *capacité résiduelle fonctionnelle* totale et unilatérale s'est abaissée à la suite de la paralysie du phrénique chez 1 des 10 malades n'ayant été soumis qu'à la paralysie phrénique seule, 9 des ces patients dont 3 avaient été soumis aux déterminations unilatérales n'ont montré aucun changement dans la ventilation moyenne du poumon comme conséquence de l'intervention qu'ils avaient subie. D'autre part la capacité résiduelle fonctionnelle était réduite chez chacun des 7 patients pour lesquels la paralysie temporaire du phrénique avait été associée à la création d'un pneumopéritoine. Des déterminations unilatérales ont démontré une action sélective du côté de l'écrasement phrénique chez 6 de ces 7 patients.

Le volume résiduel était rarement réduit par l'écrasement phrénique seul, mais en général, il s'abaissait quand le pneumopéritoine lui était associé.

La *capacité vitale*, la contribution unilatérale à la *ventilation* et la *consommation d'oxygène* ainsi que la *capacité de ventilation maxima* était habituellement réduites de façon significative après l'opération.

L'écrasement phrénique provoquait chez tous les malades une perte de fonction. Ce n'est que chez ceux ayant été soumis au pneumopéritoine qu'un effet favorable sur la ventilation moyenne du poumon a été démontré.

Acknowledgments

The nitrogen analyses and most of the studies of bilateral ventilation were performed by trained Navy corpsmen, whose skillful assistance is gratefully acknowledged. These included John Briggs, Henry Haddad, Roy Johannessen, Richard Kreinik, Joseph Malin, Robert Obser, Ernest Picknally, Robert Rauf, and Edward Stanislewski.

The charts were drawn by Mrs. Louise Ray.

The patients were under the care of Captain Edgar Riven and Captain John A. C. Gray, without whose interest and cooperation these studies could not have materialized.

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INTERMITTENT POSITIVE PRESSURE BREATHING IN PATIENTS WITH CHRONIC BRONCHOPULMONARY DISEASE[1]

A Comparative Evaluation of the Effects of Intermittent Positive Pressure Breathing Alone, Oxygen-Nebulized Bronchodilator Alone, and the Two Procedures Together

NANCY WU, WILLIAM F. MILLER, ROBERT CADE, AND PAUL RICHBURG

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INTRODUCTION

Intermittent positive pressure breathing has been employed frequently in recent years for the treatment of conditions associated with disturbances in respiration. Numerous reports based on clinical observations and physiologic measurements suggest that both subjective benefit and functional improvement have been obtained following the use of the procedure (1–13). However, except for those instances in which intermittent positive pressure breathing was used solely as a resuscitative procedure, the simultaneous nebulization of a bronchodilator drug has been employed along with it. Therefore, the data reported do not make clear whether the beneficial effects are a result of the positive pressure alone or are a consequence of bronchodilator drugs, or both.

Segal and associates (14) suggested, from vital capacity measurements obtained in five asthmatic patients, that the combination of intermittent positive pressure breathing plus a nebulized bronchodilator produces a greater and more lasting improvement in breathing than that which could be obtained from the positive pressure breathing procedure alone or from the bronchodilator alone.

Recently Fowler and associates (15) made short-term observations on groups of ambulatory patients without severe ventilatory insufficiency who were treated in such a way as to make possible a comparison of the results of several combinations of therapy, as judged by improvement of pulmonary function. Their data indicated that oxygen-aerosolized Isuprel[2] was superior to oxygen-intermittent positive pressure breathing alone, and was as effective as the combined use of oxygen-positive pressure and nebulized Isuprel[2]. Nevertheless, they recommended the use of intermittent positive pressure breathing to improve pulmonary ventilation in other patients who have severe impairment of pulmonary function with marked hypoxemia and retention of carbon dioxide.

More recently Segal and associates (16) reported a study of the comparative effects of inspiratory intermittent positive pressure breathing without simultaneous bronchodilator, and inspiratory intermittent positive pressure with nebulized bronchodilator in one group of patients. They stated that inspiratory intermittent positive pressure breathing alone caused a decrease of ventilatory function, whereas inspiratory positive pressure with simultaneously administered

[1] From the Cardio-Pulmonary Laboratory of the Department of Internal Medicine of Southwestern Medical School of The University of Texas, Dallas; and the Cardio-Respiratory Laboratory of the Veterans Administration Hospital, McKinney, Texas.

[2] α-(Isopropylaminomethyl) protocatechuyl alcohol.

nebulized bronchodilator produced striking improvement in all ventilatory functions studied.

They further compared inspiratory intermittent positive pressure breathing with nebulized bronchodilator, and nebulized bronchodilator administered only by hand nebulization in another group of patients, and concluded that nebulization by inspiratory positive pressure was superior. The methods employed in these studies are not clearly defined, and therefore it is difficult to determine the significance of the changes reported.

The purpose of the present study was twofold: to distinguish the effects of oxygen-intermittent positive pressure breathing alone from those of the bronchodilators simultaneously administered; and to compare the effects of oxygen-nebulized bronchodilators alone with those of nebulized bronchodilators administered by intermittent positive pressure breathing.

MATERIALS AND METHODS

This experiment was designed so that each patient would serve as his own control for all studies. Twenty-four patients, ranging in age from twenty-five to seventy-three years (average, fifty-five), were studied. Their clinical diagnoses, summarized in table 1, included chronic pulmonary fibrosis and emphysema, asthmatic bronchitis, cor pulmonale, and mitral stenosis with previous cardiac failure.

Ventilatory function was evaluated by measuring the dynamic lung volume and its subdivisions, the timed expiratory capacities, and maximum breathing capacities, using a 13 L. Collins Respirometer (carbon dioxide absorber and valves removed in order to decrease resistance to air flow). Since patients with bronchial obstruction associated with chronic bronchopulmonary diseases show a tendency to trap air in the lung following deep inspiration, the sum of the expiratory reserve volume plus the inspiratory capacity, which is designated as the inspiratory vital capacity, is usually larger than the conventional expiratory vital capacity. The difference between these two values provides some indication of the degree of air trapping and the severity of the ventilatory obstruction.

In a small number of patients, functional residual capacity and the index of intrapulmonary gas distribution were determined but, since no consistent changes could be noted, this part of the study was not continued. Initially, measurements of resting ventilation, respiratory rate, and oxygen removal rate were also made but, since no significant changes were observed, these measurements were also discontinued.

Control pulmonary function studies were performed before each therapeutic procedure. Studies were then repeated twenty minutes after, and one hour after, each of the following procedures:

1. Administration of intermittent positive pressure breathing with oxygen by

TABLE 1

DIAGNOSES OF 24 PATIENTS WITH BRONCHOPULMONARY DISEASE IN WHOM THE EFFECTS OF INTERMITTENT POSITIVE PRESSURE BREATHING AND OXYGEN BRONCHODILATOR WERE EVALUATED

Diagnosis	Number of Cases
Chronic pulmonary emphysema and fibrosis	12
Asthmatic bronchitis or bronchial asthma	6
Post-empyema with pulmonary fibrosis	1
Pulmonary emphysema and fibrosis with cor pulmonale	5
Mitral stenosis with previous cardiac failure	1

FIG. 1. Oxygen-bronchodilator nebulization technique. This photograph illustrates the apparatus used to administer oxygen bronchodilator aerosol by the expiratory-inspiratory-pause method of inhalation described in the text.

Bennett, flow-sensitive, apparatus (Model TV-2P) using 10 cm. of water inspiratory pressure for ten minutes. Alevaire[3] was used in the nebulizer to avoid excessive drying of the tracheobronchial tree during the inhalation period.

2. Nebulization of Aerolone Compound[2],[4] using a DeVilbis No. 40 nebulizer with an open side vent attached to an OEM oxygen mask and administered by oxygen at 6 to 7 liters per minute flow for ten minutes. The apparatus used is illustrated in figure 1. The technique of inhalation which was emphasized in this study is described in detail in a previous publication (17). The technique requires complete exhalation preceding each inhalation; inhalation is done slowly and is followed by a period of two or three seconds of breath holding. This technique was referred to as the expiratory-inspiratory-pause method of nebulization therapy, and has been shown to be more effective than the conventional successive inspiration method. The amount of bronchodilator drug used was the same in both this and the subsequent step of the experiment.

[3] Triton-WR-1339, a complex alkylaryl polyether alcohol in a concentration of 0.125 per cent, in combination with sodium bicarbonate, 2 per cent, and glycerin, 5 per cent.

[4] Supplied by Eli Lilly Company, Indianapolis, Indiana.

TABLE 2

MEAN VALUES OF LUNG VOLUMES, MAXIMUM BREATHING CAPACITIES, AND TIMED EXPIRATORY CAPACITIES IN 24 PATIENTS WITH BRONCHOPULMONARY DISEASE

	Measured Value (Liters)	Per Cent of Predicted Normal	Timed Expiratory Capacity / Expiratory Vital Capacity × 100 (Per Cent)
Inspiratory vital capacity	3.19	82	
Expiratory vital capacity	2.90	75	
Inspiratory capacity	2.00	81	
1-second expiratory capacity	1.27		44
2-second expiratory capacity	1.72		59
3-second expiratory capacity	2.04		70
Maximum breathing capacity, L./min.	48.00	34	

3. The administration of oxygen intermittent positive pressure with nebulized Aerolone Compound® using 10 cm. of water inspiratory pressure for ten minutes.

Each stage of the study was done in the above order. Sufficient time interval (two to three hours or more) after completion of each group of studies was allowed for the ventilatory functions to return to the control values and to eliminate the factor of fatigue of the patient. Control observations were obtained just before each treatment to ensure the absence of residual effects of the previous treatment. However, since statistical analysis revealed that the subsequent controls were the same or not significantly different from the original control, only the data from the first control observations were included in the comparisons. One-hour post-treatment studies were omitted when oxygen-intermittent positive pressure breathing was used alone, since the immediate functional change was small and variable.

RESULTS

The patients represented a group selected at random from the hospital wards and outpatient clinics during the period covered by this study. These were pa-

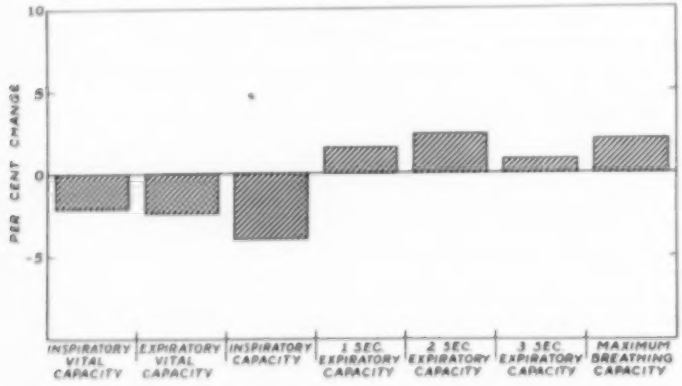

FIG. 2. Effect of oxygen-intermittent positive pressure breathing on ventilatory function in 24 patients. These changes were not found to be significant.

tients with ventilatory insufficiency ranging from moderately severe to severe, as shown in table 2. The mean values for the control periods are listed and reveal that the maximum breathing capacity was only 34 per cent of predicted normal, the ratio of one-second expiratory capacity to total expiratory vital capacity was only 44 per cent (normal > 75 per cent), and the total expiratory vital capacity was 2.90 liters, or 75 per cent of the predicted normal, while the inspiratory vital capacity was 3.19 liters, or 82 per cent of the predicted normal.

The administration of oxygen-intermittent positive pressure breathing alone did not produce a significant change in the group as a whole (figure 2) ($P < 0.4$

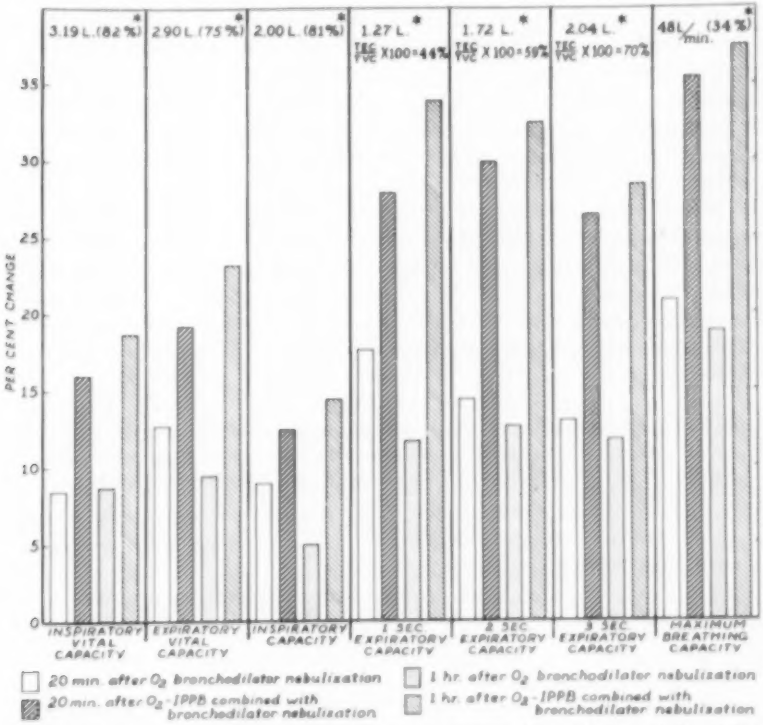
3.19 L. (82%)*
2.90 L. (75%)*
2.00 L. (81%)*
1.27 L.* TEC/TVC X100=44%
1.72 L.* TEC/TVC X100=59%
2.04 L.* TEC/TVC X100=70%
48 L./min. (34%)*
PER CENT CHANGE
0
5
10
15
20
25
30
35
INSPIRATORY VITAL CAPACITY
EXPIRATORY VITAL CAPACITY
INSPIRATORY CAPACITY
1 SEC. EXPIRATORY CAPACITY
2 SEC. EXPIRATORY CAPACITY
3 SEC. EXPIRATORY CAPACITY
MAXIMUM BREATHING CAPACITY
20 min. after O2 bronchodilator nebulization
20 min. after O2-IPPB combined with bronchodilator nebulization
1 hr. after O2 bronchodilator nebulization
1 hr. after O2-IPPB combined with bronchodilator nebulization
* Liter values represent means for entire group during the control period. Value in parenthesis is the per cent of predicted normal which the liter values represent.

FIG. 3. Comparison of the effects of oxygen-nebulized bronchodilator with those of oxygen-intermittent positive pressure breathing combined with nebulized bronchodilator twenty minutes after and one hour after.

$$\frac{\text{TEC}}{\text{TVC}} \times 100 = \frac{\text{timed expiratory capacity}}{\text{total vital capacity}}$$

Per cent change shown on the ordinate indicates per cent increase over control values. Pulmonary function studies are on the abscissa.

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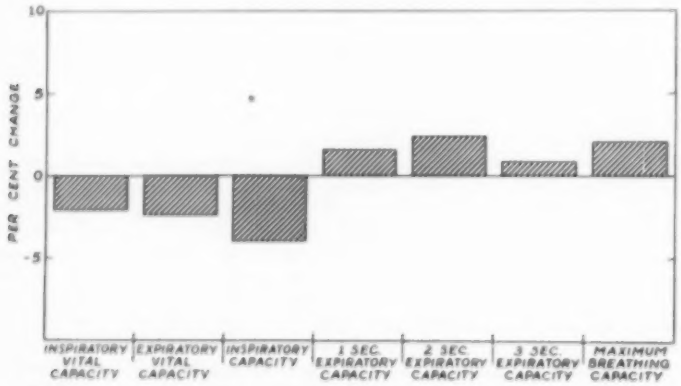

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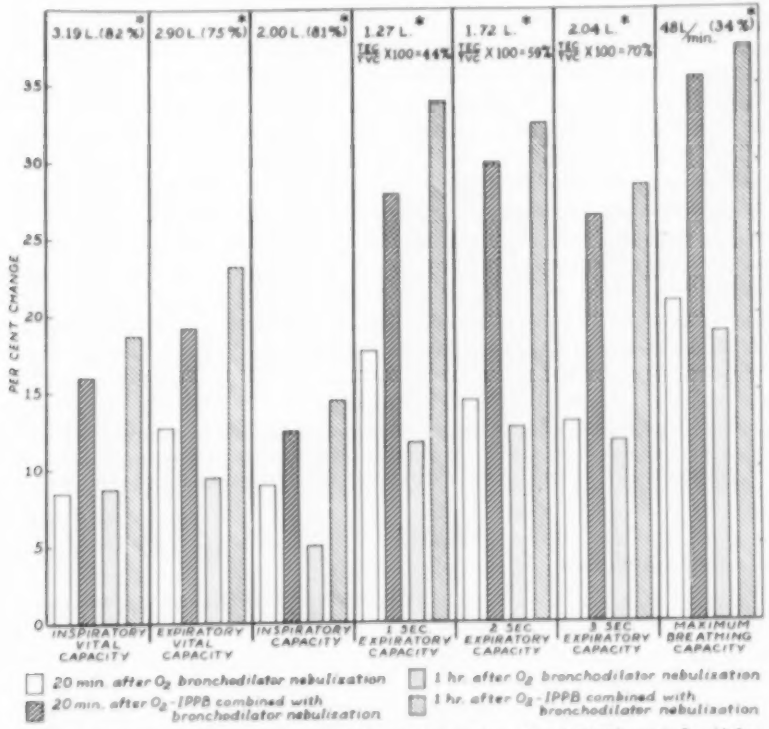
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1 SEC. EXPIRATORY CAPACITY
2 SEC. EXPIRATORY CAPACITY
3 SEC. EXPIRATORY CAPACITY
MAXIMUM BREATHING CAPACITY
20 min. after O2 bronchodilator nebulization
20 min. after O2 - IPPB combined with bronchodilator nebulization
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Per cent change shown on the ordinate indicates per cent increase over control values. Pulmonary function studies are on the abscissa.

to < 0.6). It was found, however, that the 6 asthmatic patients showed a significant decrease in the one-second expiratory capacity and maximum breathing capacity ($P < 0.02$ and < 0.05, respectively). In addition, the 5 patients with cor pulmonale in failure manifested minor, but significant, improvement in all ventilatory functions following oxygen-intermittent positive pressure treatment ($P < 0.01$ to < 0.05).

Both oxygen-nebulized bronchodilator and oxygen-intermittent positive pressure breathing combined with nebulized bronchodilator caused significant improvement of pulmonary function as shown in figure 3 ($P < 0.001$ to < 0.05). It is further noted (figure 3) that the per cent of improvement was significantly greater after oxygen-intermittent positive pressure nebulization of bronchodilator ($P < 0.02$ to < 0.001). The effects of oxygen-intermittent positive pressure plus nebulized bronchodilator were also more sustained than oxygen-nebulization of bronchodilator alone. This is also illustrated in figure 3, which reveals that each function showed a decrease one hour after oxygen with nebulized bronchodilator, whereas one hour after oxygen-intermittent positive pressure breathing with nebulized bronchodilator, ventilatory function was actually better than it was immediately after the treatment. The difference was especially apparent with respect to the improvement of expiratory vital capacity as compared with the inspiratory vital capacity, and also with respect to the marked increase of maximum breathing capacity and timed expiratory capacities.

Appraisal of cases (table 3) in which excessive secretions or severe ventilatory insufficiency were not a serious problem revealed that oxygen nebulization of

TABLE 3

EFFECTS OF OXYGEN-NEBULIZED BRONCHODILATOR AND OXYGEN-INTERMITTENT POSITIVE PRESSURE BREATHING (O_2-IPPB) COMBINED WITH NEBULIZED BRONCHODILATOR ON VENTILATORY FUNCTION TESTS IN PATIENTS WITHOUT EXCESSIVE SECRETIONS

Patient and Diagnosis	Inspiratory Vital Capacity, L.			Expiratory Vital Capacity, L.			1-Second Expiratory Capacity, L.			Maximum Breathing Capacity, L/Min.		
	Control	O_2-NB*	O_2-IPPB NB*	Control	O_2-NB*	O_2-IPPB NB*	Control	O_2-NB*	O_2-IPPB NB*	Control	O_2-NB*	O_2-IPPB NB*
1. W. A. Mitral stenosis	2.01	2.30	2.34	1.90	2.26	2.30	1.90	2.21	2.16	60	77	72
2. F. G. Post-empyema with pulmonary fibrosis	2.01	2.40	2.41	1.92	2.36	2.19	1.25	1.47	1.45	49	56	60
3. E. L. Pulmonary fibrosis	2.96	3.44	3.33	2.78	3.13	3.15	1.75	2.17	2.18	49	57	61
4. C. W. Pulmonary emphysema with cor pulmonale	3.07	3.45	3.18	3.02	3.32	3.09	1.32	1.43	1.21	51	62	54
5. B. C. Pulmonary fibrosis with cor pulmonale	1.49	1.70	1.97	0.59	1.15	1.15	0.15	0.29	0.21	7	9	11
Mean	2.31	2.66	2.65	2.04	2.44	2.37	1.23	1.51	1.44	43	52	52
Mean difference		0.01			0.07			0.07			0	

* Nebulized bronchodilator.

bronchodilator by the method of inhalation described above was nearly, or equally, as effective as nebulization of bronchodilator with oxygen-intermittent positive pressure breathing. However, when severe ventilatory insufficiency and excessive secretions result in airway obstruction and ineffective alveolar ventilation, which was true in most of the patients, dissemination of bronchodilator drugs was more effectively accomplished by oxygen-intermittent positive pressure nebulization.

DISCUSSION

It is apparent from the results of this study that the greatest and most prolonged improvement of ventilatory function in patients with severe chronic bronchopulmonary disease and obstructing secretions was obtained with oxygen nebulization of bronchodilator drug combined with intermittent positive pressure breathing. Aside from the well-known effects of bronchodilator drugs, in these patients the effects of oxygen-intermittent positive pressure breathing are twofold. First, the intermittent positive pressure apparatus produced increased depth of respiration, some dilatation of the small airways, and more uniform ventilation of the air spaces than usually resulted from the subject's own breathing efforts (8). Therefore, the simultaneously administered bronchodilator drug is more uniformly distributed and optimal effect is obtained. Second, oxygen-intermittent positive pressure with nebulized bronchodilator promotes bronchial drainage, since the rhythmic inflation and deflation of the lungs which usually results from the use of this method tends to assist in propulsion of secretions out of the bronchial tree (18). This is accomplished by virtue of the rapid release of the inspiratory pressure at the beginning of expiration, with a resulting high velocity expiratory air flow. Thus, obstruction due to mucous plugs might be reduced or eliminated.

The effects described above undoubtedly played a significant role in this series and would tend to explain the greater and more sustained improvement in functions obtained by the use of oxygen-intermittent positive pressure combined with nebulized bronchodilator as compared with oxygen-nebulization of bronchodilator without intermittent positive pressure breathing.

On the other hand, in those patients who are able to cooperate by using special breathing techniques, no significantly greater improvement in function was observed with the addition of intermittent positive pressure to the oxygen nebulization procedure (table 3). Even in this group of subjects who did not suffer from obstructing secretions and severe ventilatory insufficiency, however, the oxygen-intermittent positive pressure bronchodilator nebulization produced improvement in ventilatory function which was of longer duration than oxygen-bronchodilator nebulization without intermittent positive pressure. This may have been a consequence of improved distribution of the bronchodilator resulting from intermittent positive pressure in excess of that which could be achieved by proper breathing methods alone.

In either case, the effectiveness of nebulized bronchodilator drugs depends to a large extent upon their distribution in the tracheobronchial tree. When using

the customary methods of nebulization without intermittent positive pressure, distribution is determined by the effectiveness of the patient's inspiratory efforts in carrying the drug into the lung. Airway obstruction, increased functional residual capacity, and diminished alveolar ventilation are among the more important factors known to prevent adequate distribution and dissemination of the bronchodilator drug. Improved distribution can presumably be achieved by deep breathing (18). However, in most patients with chronic obstructive disorders, deep breathing favors air trapping and results in progressive decrease of inspiratory capacity and increase of functional residual capacity. Thus, the effectiveness of bronchodilator drugs is reduced, since a comparatively small amount of nebulized material is distributed in a relatively large air space. This disadvantage would be overcome, in part at least, by the method used in this study and described elsewhere as the expiratory-inspiratory-pause method of nebulization (17). A somewhat similar use of an inspiratory pause was also recommended by Fowler and associates (15). When oxygen-intermittent positive pressure breathing was used without bronchodilator aerosol, the decrease in one-second expiratory capacity and maximum breathing capacity which was found in patients with bronchial asthma confirms similar findings obtained by Segal and associates (16). This effect may be due to reflex bronchospasm from the stimulation of pulmonary stretch reflexes by the greater distention of the lung induced by the intermittent positive pressure. Such bronchospasm was opposed, however, by the effect of the bronchodilator drugs when administered with oxygen-intermittent positive pressure breathing.

The suggestion by Segal that the decreased function in such patients was due to the reflux of blood into the pulmonary tissues and fatigue is unsupported by evidence from the data presented (16). Moreover, in the present studies, the evidence points not to a restrictive change, which might be expected from either fatigue or a reflux of a larger volume of blood into the pulmonary tissue, but to an obstructive change such as would occur with bronchospasm. The element of fatigue was eliminated in the present study by performing the oxygen-intermittent positive pressure phase of the study first and allowing adequate time between each part of the study so as specifically to avoid fatigue.

In patients who had pulmonary congestion associated with evidence of heart failure, the increased function observed with the use of oxygen-intermittent positive pressure alone may have resulted from an increase of pulmonary compliance.

The pressures and the duration of treatment used in the present study do not necessarily constitute a recommendation of this specific regimen for routine use. Pressures, medications, and duration of the treatment to be used with inspiratory intermittent positive pressure breathing apparatuses is a special problem which requires individualized consideration and is beyond the scope of this report.

Since this study was completed, the present writers have had limited experience with the Emerson[5] inspiratory positive pressure respiratory assistor, as well

[5] J. H. Emerson Company, Cambridge, Massachusetts.

as the Haliburton[6] unit, both of which are entirely satisfactory units for such treatments. Each has certain minor advantages and disadvantages under given circumstances, but again a discussion of the technical aspects of these instruments is beyond the scope of this study.

SUMMARY

Studies designed to evaluate the beneficial effects of intermittent positive pressure breathing in pulmonary disease have been complicated by the simultaneous administration of bronchodilators. This investigation was undertaken in an effort to distinguish the effects of intermittent positive pressure breathing alone from those of the bronchodilator agents administered simultaneously and to compare the effects of nebulized bronchodilator alone with those of nebulized bronchodilator administered by intermittent positive pressure breathing.

The results of studies revealed that:

1. Oxygen-intermittent positive pressure breathing alone, except for the immediate effects of the oxygen administered, is of no value in chronic bronchopulmonary disease.

2. Oxygen-intermittent positive pressure breathing enhances the effectiveness of simultaneously administered bronchodilator aerosol, as a result of improved distribution of the inhaled bronchodilator and better evacuation of bronchial secretions.

3. Whereas oxygen-intermittent positive pressure breathing, combined with nebulized bronchodilator, offers significant acute benefits when excessive secretions or severe ventilatory insufficiency is a major problem, oxygen-nebulization of bronchodilators by improved breathing techniques, but without intermittent positive pressure breathing, yields equal results in most other instances.

SUMARIO

La Respiración Positiva Intermitente bajo Presión en los Sujetos con Enfermedades Broncopulmonares Crónicas: Justipreciación Comparativa de los Efectos de la Respiración Positiva Intermitente bajo Presión Sola, del Broncodilatador con Oxígeno Nebulizado Solo y de la Respiración Positiva Intermitente bajo Presión Unida al Broncodilatador Nebulizado

Los estudios destinados a valuar los efectos beneficiosos de la respiración positiva intermitente bajo presión se han visto complicados por la administración simultánea de broncodilatadores. Emprendióse esta investigación con mira a diferenciar los efectos de la respiración positiva intermitente bajo presión sola de los broncodilatadores administrados simultáneamente y a comparar los efectos del broncodilatador nebulizado solo con los del broncodilatador administrado con la respiración positiva intermitente bajo presión.

Los resultados de los estudios revelaron que:

1. La respiración positiva intermitente de oxígeno bajo presión sola, aparte de los efectos inmediatos del oxígeno administrado, no posee valor en las afecciones broncopulmonares crónicas.

2. La respiración positiva intermitente de oxígeno bajo presión realza la eficacia del aerosol broncodilatador administrado simultáneamente, a consecuencia de la mejor distribución del broncodilatador inhalado y la mejor evacuación de las secreciones bronquiales.

[6] Duncan Oxygen Therapy Company, Duncan, Oklahoma.

3. En tanto que la respiración positiva intermitente de oxígeno bajo presión, combinada con el broncodilatador nebulizado, ofrece importantes beneficios agudos cuando el exceso de secreciones o la grave insuficiencia ventiladora constituyen un problema saliente, la nebulización de broncodilatadores con oxígeno por medio de técnicas respiratorias perfeccionadas, pero sin respiración positiva intermitente bajo presión, da resultados iguales en la mayor parte de los demás casos.

RESUME

Respiration sous pression positive intermittente chez les malades atteints d'affections broncho-pulmonaires chroniques: Evaluation comparée des effets produits par la respiration sous pression positive intermittente seule, par les bronchodilatateurs nébulisés par l'oxygène seuls et par la respiration sous pression positive intermittente plus l'aérosol bronchodilatateur

Les études effectuées dans le but d'évaluer, dans les affections pulmonaires, les effets favorables de la respiration sous pression positive intermittente, ont été rendues plus complexes par l'adjonction simultanée de broncho-dilatateurs. L'investigation actuelle a été entreprise pour essayer d'établir une distinction entre les effets de la respiration sous pression positive intermittente seule et ceux qui résultent de l'administration simultanée de broncho-dilatateurs, et aussi pour comparer les effets du broncho-dilatateur nébulisé seul avec ceux produits par le broncho-dilatateur nébulisé administré par le procédé de respiration sous pression positive intermittente.

Les résultats de l'étude ont révélé que:

1. L'inhalation d'oxygène sous pression positive intermittente seule, sauf l'effet immédiat de l'oxygène administré, est sans valeur dans l'affection broncho-pulmonaire chronique.

2. L'inhalation d'oxygène sous pression positive intermittente augmente l'efficacité de l'aérosol broncho-dilatateur administré simultanément, comme conséquence d'une meilleure distribution du bronchodilatateur inhalé et d'une meilleure évacuation des sécrétions bronchiques.

3. Ce qui demeure un problème majeur, c'est de déterminer si l'inhalation d'oxygène sous pression positive intermittente, associée à la nébulisation d'un broncho-dilatateur, offre des avantages appréciables en présence de mucosités particulièrement abondantes ou d'une insuffisance marquée de la ventilation; car des résultats égaux sont obtenus dans la plupart des autres cas par la nébulisation de broncho-dilatateurs par l'oxygène à l'aide de techniques d'inhalation améliorées, la respiration sous pression positive intermittente étant exclue.

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ISOLATION, IDENTIFICATION, AND CLASSIFICATION OF PROTEINS OF TUBERCULIN AND THE TUBERCLE BACILLUS[1, 2]

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INTRODUCTION

Preparations of Old Tuberculin vary in their potencies and, therefore, are not dependable for use in the diagnostic skin test for tuberculosis. For this reason attempts by many investigators, beginning with Koch himself, have been made to isolate the active fraction which has been shown to contain the specific activity (1, 2). A protein product practically free of all the impurities in the culture filtrate, including polysaccharide and nucleic acid, has been adopted as the International Standard (3) for such preparations.

However, when this product was studied by the newer physicochemical methods, it was found to be a mixture of proteins with different electrophoretic properties. The isolation of these proteins, therefore, became a goal. As heat modifies protein, it was necessary to make fractionations on unheated tuberculin. At least three different proteins were isolated (4), and they were found to have different relative potencies when tested in comparable dosage based on their nitrogen contents.

Before any one of these proteins could replace the present PPD-S for diagnostic purposes, it would be necessary to establish the ability of obtaining it with reproducible composition as well as potency. Moreover, its potency should be at least as great as that of the PPD-S in persons with clinically significant tuberculosis. The present study is an attempt to survey the chemical and biological properties of many protein fractions isolated from tuberculins of different strains, on different media and by different methods, and to determine their reproducibility.

A study such as this has also the possibility of detecting protein fractions which have significant potencies for persons with hypersensitivities due to different causes, as suggested by Palmer (5).

MATERIALS

Many different tuberculin protein preparations prepared in the past were utilized. In general the preparations were isolated by the alcoholic low-temperature procedure which was previously described (4) and which was utilized in the preparation of lot No. 109, one of the preparations discussed in this paper. Details on the preparation of fractions will be limited to the last three lots of tuberculin, viz., lots Nos. 107, 108, and 109.

[1] From the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[3] Fellow of the International Branch of the United States Public Health Service.

[4] Deceased, December 4, 1954.

Preparations Nos. 107 and 108 were made from two batches of raw culture filtrate of the H37Ra strain grown on Wong-Weinzirl medium at pH 6.5 in the Research Division of Eli Lilly & Co. by Dr. William B. Sutton. Lot No. 107 consisted of 4,250 ml. of filtrate of a 22-week-old culture and 29,670 ml. of an 11-week-old culture. Lot No. 108 was the filtrate of 30,000 ml. of a 13-week-old culture. The bacilli of both lots were filtered off alive, and the filtrates were refiltered through the Seitz filter. They were then distributed in 250 ml. amounts into bottles and lyophilized. Lot No. 107 did not dry well and was pooled and refiltered before shipping to this laboratory (1,800 ml.). Lot No. 108 did dry well and was shipped in the lyophilized form. The fractionations of these two lots are given below.

Lot No. 109 was prepared in this laboratory from the DT strain of bacillus grown on Long's synthetic medium. The procedure for its fractionation is also given below.

Fractionation of Tuberculins from Lots Nos. 107, 108, and 109

Lots Nos. 107 and 108: Lot No. 107 was processed in several batches, all essentially by the same process. Lot No. 108 was put into solution with water in a volume of 1,200 ml. Both lots were processed in the following manner.

The original solutions, both of which had a pH of 5.8, were clarified by centrifugation in the refrigerated Spinco Preparative Ultracentrifuge[a] at 20,000 to 30,000 r.p.m. for forty minutes. A dark brown jelly-like aggregate and a creamy granular sediment (probably inorganic salts) were removed and discarded, leaving a crystal clear deep reddish-brown solution.

In the case of lot No. 108 the solution was washed at 3°C. by ultrafiltration with approximately 3,500 ml. of water on four gun-cotton membranes (11 per cent) (6) in order to remove a large amount of the salts and medium constituents. Another brown gel deposited on the membranes, was removed from the washed solution by centrifugation at 40,000 r.p.m. for forty-five minutes, and discarded. The solution was again filtered through paper. From here on this solution was treated in the same way as the original clarified solution of lot No. 107. The method was as follows.

The solutions were brought to pH 4.0 by the gradual addition of acetic acid during constant stirring. The freely aggregating precipitates were collected by centrifugation at 20,000 to 30,000 r.p.m. for forty minutes. They were washed with acetate buffer pH 4.0, centrifuged, redissolved in phosphate buffer pH 7.6, dialyzed against this buffer, and finally again centrifuged at 40,000 r.p.m. for forty minutes to free them of a deep brown gel. The clear solutions were filtered through the Seitz filter and stored in sterile condition at −6°C. These fractions were called 107 I and 108 I and corresponded to the proteins previously (4) designated as C protein fractions.

The supernatants from the pH 4.0 precipitates were saturated with solid ammonium sulfate while stirring and allowed to stand overnight at 3°C. The precipitates were then removed by centrifuging at 20,000 to 30,000 r.p.m. for thirty-five to forty minutes at 5°C., washed twice with saturated ammonium sulfate solution, suspended in a small amount of phosphate buffer pH 7.6, $\mu = 0.1$, and dialyzed (in the case of lot No. 107) or washed free of ammonium sulfate by ultrafiltration (in the case of lot No. 108). The dialyzed or washed solutions were then filtered through the Seitz filter and stored as sterile solutions at −6°C. These fractions were designated 107 II + III and 108 II + III, and corresponded to the A and B types of protein described previously (4). However, there was very little B type of protein in either of these fractions, and so no attempt was made to separate the A and B proteins as was originally planned.

The supernatants from the saturated ammonium sulfate precipitates were of deep amber color and had a pH 4.0. They were concentrated and washed with water by ultrafiltration at 3°C. until free of ammonium sulfate and refiltered through the Seitz filter. These fractions

[a] The writers wish to thank Sharp and Dohme Co. for contributing the Spinco Preparative Ultracentrifuge.

were called 107 IV and 108 IV and, since they contained a large amount of polysaccharide, they were called glycoproteins, although it has not yet been conclusively established that they are true compounds of protein and carbohydrate.

Lot No. 109: This lot was processed after it was found that very little of the B type of protein was present in either of lots Nos. 107 or 108, which had been made from the H37Ra strain. A review of earlier work showed that the B type of protein had been isolated chiefly when the DT strain was used.

This lot (No. 109) was made by growing the DT strain on Long's synthetic medium for nine weeks. The bacteria were filtered off alive through filter paper and then through the Seitz filter, yielding 17,175 ml. of filtrate. This solution was concentrated by ultrafiltration at 4°C. to 164.5 ml., and acetate buffer was added with stirring until the pH was 4.6. The precipitate (109 I) was washed three times with buffer pH 4.6 and then suspended in 0.9 M acetic acid pH 2 to 3. The soluble portion (109 Ia) was centrifuged off, dialyzed against phosphate buffer pH 7.8, and clarified by high-speed centrifugation at 5°C. The portion insoluble at pH 2 to 3 (109 Ib) was suspended in phosphate buffer pH 7.8, dialyzed against this buffer, which effected solution, and clarified by high-speed centrifugation at 5°C. Both of these fractions represented C type proteins.

The portion of the original filtrate soluble at pH 4.6 was further fractionated by means of alcohol at low temperature by the technique used in previous studies (4). The solution was first concentrated by ultrafiltration to 77 ml., and then absolute alcohol was added until a 30 per cent concentration was obtained. The precipitate obtained was divided into a small portion insoluble at pH 7.5 (109 Va), which by the previous scheme (4) would probably be polysaccharide, and a larger soluble portion. The soluble portion was precipitated at pH 4.6, giving the B type of protein, and then this precipitate was suspended in and dialyzed against phosphate buffer pH 7.6. A considerable amount of this dialyzed solution centrifuged down at 30,000 r.p.m. at 5°C. as a pink pellet, which was finally put into solution with dilute sodium hydroxide and neutralized with acetic acid, giving a reddish solution. It was filtered through the Seitz filter and was called 109 IIa. The supernatant, containing the more soluble portion (109 II), was also filtered through the Seitz filter. Both were considered to be B type proteins, since they were soluble at pH 7.6 in 30 per cent alcohol but insoluble at pH 4.6.

The portion of the original solution still soluble at pH 4.6, even after the addition of 30 per cent alcohol, was precipitated further by the addition of more alcohol until the concentration was 70 per cent. This precipitate, which represented the A type of protein, was removed by centrifugation at 30,000 r.p.m., suspended in and dialyzed against buffer pH 7.4, and clarified again by centrifugation and filtration through the Seitz filter. It was called 109 III.

The entire remaining solution, soluble at pH 4.6 in 70 per cent alcohol, was then concentrated by ultrafiltration, washed with phosphate buffer pH 7.6, and clarified by high-speed centrifugation and final filtration through the Seitz filter. It was called 109 IV and was probably mainly glycoprotein. It was almost identical in its electrophoretic pattern with the fraction 100 IV previously isolated (7) in the same way from the H37 strain.

Bacillary bodies of Lot No. 109: Some of the moist DT bacilli were extracted by means of urea as described earlier (8). An amount of solid urea equal to twice the weight of the moist bacilli was added. The whole was stirred and kept in the incubator room at 37°C. for seventy-two hours with frequent stirring. The pasty mixture at first became very cold and later appeared to liquefy. Water, equivalent to the weight of the bacilli, was then added, and the mixture was filtered through paper and then through the Seitz filter. The clear supernatant was concentrated at 4°C. by ultrafiltration after the addition of an equal volume of water and was finally washed free of urea with phosphate buffer pH 7.6, $\mu = 0.02$. Acetate buffer was then added until the pH was 4.0. The resulting precipitate was centrifuged off, suspended in phosphate buffer pH 7.6, dialyzed against this buffer, and clarified by filtration through the Seitz filter. This fraction was called 109 B Id. Some of this precipitate was suspended in acetic acid of pH 2.4, and the portion which went into solution was

dialyzed against phosphate buffer. It was then filtered through the Seitz filter and was called 109 B Ia.

The material originally soluble at pH 4.0 was then concentrated by ultrafiltration, dialyzed free of urea against phosphate buffer pH 7.6, and filtered through the Seitz filter. It was designated 109 B II.

Methods of Analysis and Test

Nitrogen was measured by the Markham method (9), and protein was calculated from this by the use of the factor 6.25. *Nucleic acid* was determined by the Dische diphenylamine (10) method, using pure desoxyribonucleic acid as standard. *Carbohydrate*, calculated as glucose, was determined by the Koehler modification (11) of the Morris anthrone method, using a ten-minute heating period.

Ultraviolet spectral absorptions were measured in the Beckman spectrophotometer.

Electrophoretic patterns were obtained with the Tiselius electrophoresis apparatus, using in all cases phosphate buffer pH 7.6, $\mu = 0.1$, at a potential gradient of 9 to 10 volts. The mobilities were recorded in units of 10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$.

Paper chromatograms were made in the ascending direction on 8- by 8-inch sheets of Whatman No. 1 paper, first in phenol and then in butyl-acetic solvents, prepared according to Berry (12). The patterns were developed by spraying with 0.25 per cent solution of ninhydrin in anhydrous acetone and then drying for four minutes at 90°C. Reducing sulfur amino acids were specifically detected by spraying other chromatograms with a solution made according to Toennies and Kolb (13) as follows: 4 ml. of 0.002 M platinochloric acid plus 0.25 ml. M potassium iodide plus 0.4 ml. of 2 N hydrochloric acid plus 76 ml. of acetone. On drying at room temperature, white to pale yellow areas appeared against a pink background in the position of the reducing sulfur compounds.

Cystine and α-ϵ-diaminopimelic acid were identified by the use of the highly sensitive and specific *mutants* of *Escherichia coli* isolated by Davis (14). The mutant No. 173-25, which requires diaminopimelic acid for growth, was seeded in agar plates made from basic medium (15) supplemented with lysine. When drops of the hydrolysates were put on these plates, growth became evident wherever the diaminopimelic acid was present. A similar technique for detecting the presence of cystine was used with the mutant No. 57-29, which requires cystine for growth, seeded in plates made from the simple basic medium.

Skin tests were made both on guinea pigs and patients. The guinea pigs used had been previously infected with the DT strain of tubercle bacillus. Comparable doses (0.005 mg. of protein based on the nitrogen contents) of the different fractions were injected intradermally in 0.1 ml. amounts simultaneously in sites which were randomized in the group of pigs tested. The identity of these sites was not known at the time of reading. Standard PPD-S was included in all series of tests. After twenty-four and forty-eight hours, the reactions were read as two diameters at right angles to each other, and the height of the reaction was estimated on a scale of 1, 2, 3, and 4. The two diameters of each reaction were then averaged and recorded in the table with the height.

The patients were from the chest clinic of the Henry Phipps Institute. Intradermal injections of 0.1 ml. amounts of comparable dilutions of the fractions were made in the volar surfaces of the forearms approximately 2 inches below the elbow. Each product was compared with the standard PPD-S, given in the opposite arm. After forty-eight hours the amount of induration of the reactions was measured as two diameters at right angles to each other, and the height of the reaction was estimated on a scale of 1, 2, 3, and 4. On alternate patients the areas were reversed, and the identity of the product used in each site was not known to the observer when the readings were made.

RESULTS

Yields

In table 1 are recorded the yields of the different fractions, calculated from refractive index determinations on the solutions, using as control the buffer

TABLE I

YIELDS OF ISOLATED FRACTIONS

Yields of Fractions from Lots Nos. 107, 108, and 109 and from Bacilli of Lot No. 109

Lot Number	Strain Used	Medium Used	Volume of Original Filtrate	Fraction	Amount of Fraction Recovered		Type of Component
					Total Grams*	Protein†	
			ml.				
107	H37Ra	Wong-Weinzirl	33,920	I	0.36	0.29	C
				II + III	1.17	1.12	A+B
				IV	10.21	5.19	Glycoprotein
				Total	11.75	6.60	
108	H37Ra	Wong-Weinzirl	30,000	I	0.47	0.52	C
				II + III	2.93	2.55	A+B
				IV	13.77	6.18	Glycoprotein
				Total	17.17	9.25	
109	DT	Long's	17,175	I a	0.15	0.05	C
				I b	0.07	0.008	C
				II	0.56	0.45	B
				II a	0.47	0.48	B
				III	3.55	1.51	A
				IV	3.67	0.44	Glycoprotein
				V	0.04	0.007	Polysaccharide
				Total	8.51	2.94	
109B	DT Bacilli	Long's	425 gm. moist bacilli	I d	0.82	0.73	
				I e	0.23		
				II	0.25	0.14	
				Total	1.40		

* Total grams based on refractive index analyses.

† Grams of protein based on nitrogen content.

solution or water against which they had been dialyzed. The refractive index increments previously found (16) were used, viz., 0.00188 for protein and 0.0014 for polysaccharide or nucleic acid. Since the 107 IV, 108 IV, 109 III, and 109 IV fractions consisted of 40 per cent or more polysaccharide or nucleic acid, an average value of 0.00164 was used as the refractive index increment for these fractions.

The protein content was estimated on the basis of nitrogen values. The difference between these protein concentrations and the total concentrations estimated on the basis of refractive indices gives a rough idea of the amount of carbohydrate present and shows that the IV fractions in all cases contained a high percentage of carbohydrate.

TABLE 2

CHEMICAL ANALYSES

Analyses of Fractions of Lots Nos. 107, 108, and 109

Type of Protein	Lot Number	Per Cent of Total as			Maximum Ultraviolet Absorption		Absorption Ratio at Maximum/Minimum
		Protein	Carbohydrate	Nucleic Acid	Wave Length	Specific Density	
A	107	98.2	0.13	0.51	280	1.44	2.03
	108	82.6	6.96	10.65	265	3.26	1.14
	108 (a)	94.6	1.73	3.62	275	3.01	1.40
	109	59.0	40.20	0.47	280	1.60	2.61
B	109	80.9	18.75	0.31	280	1.46	2.00
	109 (a)	97.4	2.26	0.30	280	0.79	1.91
C	107	99.3	0.30	0.44	275	1.04	1.68
	108	69.8	1.05	29.10	260	8.50	1.37
	109	98.0	1.30	0.74	275	2.08	1.40
A + Glycoprotein	107	59.9	39.30	0.69	270?	0.33	1.11?
	108	42.6	43.40	14.05	255	3.40	1.70
	109	41.7	56.90	1.51	280	1.59	1.64
Bacillary protein	109 (Ia)	99.8	0.03	0.16	275	0.91	1.72
	109 (Id)	97.2	1.15	1.63	270	1.61	1.14
	109 (BII)	45.9	52.80	1.29	275	1.10	1.53

Analyses

The chemical analyses recorded in table 2 give the percentage of protein, carbohydrate, and nucleic acid in each fraction and their ultraviolet absorptions. The results show that there was very little nucleic acid in the fractions obtained from lots Nos. 107 and 109, in contrast to those from lot No. 108. The high nucleic acid content of the fractions of lot No. 108 explains their high specific densities at the maximum wave lengths, and also the fact that their maximum wave lengths were at λ255 to λ265 instead of λ280, which is characteristic of protein. (See an earlier study on this subject (17).)

In the case of the A fraction of lot No. 108, purification by the method previously recommended and used in the preparation of the standard PPD-S (2), i.e., precipitation with ammonium sulfate at neutrality, did lower the nucleic acid content as well as the specific density. (*See* A-108 (*a*) in table 2.) It also caused the maximum absorption to shift to λ275 and raised the ratio of maximum to minimum absorption from 1.14 to 1.40.

The ratios of the densities at the maximum to minimum for the A or B type of proteins when they are not grossly contaminated with nucleic acid are in general approximately two or more, whereas those for the C type of protein are usually lower. This fact confirms the results previously reported (4), and, therefore, appears to be a useful method for following the separation of these proteins.

TABLE 3

DIAMINOPIMELIC ACID

Content of α ε-Diaminopimelic Acid in Protein Hydrolysates

Type of Fraction	Lot Number	Content of α ε-Diaminopimelic Acid	
		Based on Chromatograms	Based on Growth with Mutant No. 173-25
		Per Cent	
A	98	6.3	++++
A	109	3.6	+++
B	109	3.6	++
Ba	109	0	++
C	96	0.7	
C	98	0	+ (slight)
C	108	0	
GP	100	10.7	++++
GP	109	17.0	++++
BP	101	3.7	++++
BP	109 Id	0	
BP	109 BII	2.8	
PPD-S		Trace	+
OT		High	++++
α ε-Diaminopimelic acid		100.0	++++
Cystine		0	0

GP = glycoprotein.
BP = bacillary protein.

The residue fractions are shown to contain a large percentage of carbohydrate and are, therefore, called glycoproteins. The proteins contained in these fractions have very low electrophoretic mobilities, as shown in the next section, and may, therefore, be of the A type.

Two of the bacillary proteins (109 Ia and 109 Id), which were precipitated from the bacillary urea extract at pH 4.0, appear to be relatively pure proteins. They differed only in the fact that 109 Id was the whole original precipitate and 109 Ia represented only that portion of the precipitate which was soluble at pH 2.4. The analyses indicate that some purification was effected in this manner.

Identification of α-ε-Diaminopimelic Acid[6]

Amino acids were identified by means of paper chromatograms in hydrolysates of a few samples of each type of protein. In addition to many of the well-known amino acids, in some of the preparations a spot with an R_F of approximately

[6] The writers wish to thank Dr. E. Work for some α ε-diaminopimelic acid used for identification purposes.

0.03 to 0.05 in butyl-acetic solvent and of 0.092 to 0.108 in phenol was found. This is the R_F of α-ε-diaminopimelic acid, an amino acid found by Work (18) only in mycobacteria, diphtheria bacilli, and certain algae.

The intensity of this spot on chromatograms made with 4 λ of solution, containing in each case 75 γ of protein, varied with the different fractions in a descending order as follows: glycoproteins, A protein, B protein, bacillary protein. None or only a trace was found in the C protein. When the spots were measured by the densigraph[7] and the areas of the density curves measured with a planimeter, the approximate results given in table 3 were obtained.

A number of sulfur amino acids, such as cystine, cysteine, cysteic acid, and cystathionine, give spots in close proximity, but the only one to appear in the exact position of α-ε-diaminopimelic acid under the conditions in these experiments is the sulfur amino acid lanthionine. Therefore, fresh chromatograms of the fractions were sprayed with the platinum chloride solution, which gives bleached spots in all positions of sulfur amino acids. Since the spot under question did not bleach, the original interpretation that it represented α-ε-diaminopimelic acid was justified.

Further confirmation of the presence of this amino acid in the different fractions in the relative concentrations given above was obtained by use of the mutants[8] of *Escherichia coli* of Davis (14). The estimations of the amounts of growth obtained on plates of his minimal media (15) seeded with the No. 173-25 strain, which is specific for α-ε-diaminopimelic acid, are listed in table 3. The results show that α-ε-diaminopimelic acid was present in greatest concentration in the glycoprotein fractions. Less was present in the A protein and bacillary fractions and still less in the B protein and PPD-S. It occurred only to a very slight extent in the C protein.

A similar type of experiment utilizing the No. 57–29 mutant, which is specific for cystine, showed that this amino acid was not present in two glycoprotein fractions. Therefore, the absence of cystine shown by this experiment as well as the absence of sulfur amino acids with low R_F values, which was demonstrated above in the paper chromatograms, gives strong support to the conclusion that the spot under investigation was diaminopimelic acid, as was indicated by the ninhydrin chromatograms and the mutant experiments.

Electrophoretic Properties

A summary of the electrophoretic mobilities of the fractions isolated previously as well as of those whose fractionations are given in detail in this paper (Lots Nos. 107, 108, and 109) is presented in table 4. The mobility of the main component in each diagram is italicized. Largely on the basis of these mobilities and their chemical properties the fractions were designated as A, B, C, or glycoprotein. The ranges for the mobilities of the different groups of proteins are given in table 5. The different solubilities of these fractions also indicate that they are different substances. The purest preparations in each group showed a single

[7] The writers wish to thank Arthur H. Thomas for the densigraph used in these studies.
[8] The writers wish to thank Dr. B. Davis for the mutants used in this study.

TABLE 4

IDENTIFICATION OF PROTEINS BY ELECTROPHORESIS

Electrophoretic Mobilities of Fractions

Lot Number	Strain of Tubercle Bacilli Used	Predominating Protein	Mobilities × 10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$								
			0.8–1.8	1.9–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–11.0	12.0–18.0
90	DT	A	s	s		*4.7*					
		C					*5.6*		s		
93	H37	A + GP	*0.9*	*1.9*			s				
		C						*6.4*		s	
94	DT	A + B			s	*4.7*			*7.6*		
		C						*6.3*			
95	BCG	A + B		s		*4.3*	*5.9*		s		
		C							*7.3*		
96A	A33	A	s		*3.5*		s				
		B			s			*6.2*		s	
		C			s		*5.5*			m	s
96B	A33	A	s	*2.8*		*4.1*	s			s	
		A + B	s		*3.5*		*5.9*				
		C	s		s			*6.1*		s	
97	DT	A + GP	s	*2.3*	*3.8*			s			
		B	s		s		*5.6*		s		
98	DT	A + GP	s	*2.1*	*3.4*		s				
		B	s		s			*6.0*		s	
		C						*6.4*		s	
		B(a)				s		*6.4*	*7.4*		
100	H37	A	s		*3.7*			s			
		A	s	*2.2*	s						
		A + GP	*1.1*	s	s			s			
		C						*6.6*		s	
107	H37Ra	A				*4.1*	s				
		A + GP	*0.9*	m							
		C			s				*7.4*	s	
108	H37Ra	A		s	*3.8*						s
		A + GP	*1.3*		s						s
		C				s			*7.4*	s	*17.5*
		A(a)			*3.9*						
109	DT	A	*0.8*	*2.5*		s					
		A + GP	*1.0*		s	s	s				
		B	s			s		*6.9*			
		C							*7.5*	s	
		B(a)						*6.5*			
84	DT	A + B			*3.6*			*6.4*			
		C					*5.5*				
Bacillary Fractions											
101 B	H37Rv		s					*6.0*			s
102 B	H37Ra		s				*5.8*				s
109 B	DT	II	*1.0*		*3.5*				*7.3*		
		Ia				s		*6.9*			
		Id				s		*6.5*			

s = small amount of a component with indicated mobility.

m = medium amount of a component with indicated mobility.

GP = glycoprotein.

(a) = purified fraction.

TABLE 5

CLASSIFICATION OF PROTEINS BY THEIR PROPERTIES

Ranges of Mobilities and Solubilities

Type of Protein	Mobilities $\times 10^{-5}$ $cm.^2 volt^{-1} sec.^{-1}$	Solubility Properties
C	−5.5 to −7.5	Insoluble at pH 4.0
B	−5.6 to −7.6	Soluble at pH 4.0 unless 30 per cent alcohol is present.
A	−1.9 to −4.7	Soluble at pH 4.0 unless 70 per cent alcohol is present.
Glycoprotein	−0.9 to −1.3	Soluble at pH 4.0 and in 70 per cent alcohol.

component approaching electrophoretic homogeneity, and the patterns of these fractions are given (figures 1 and 2).

The electrophoretic diagrams of the fractions of lot No. 108 confirmed the chemical analyses in showing the presence of a small fast component with the mobility of nucleic acid or a nucleoprotein in both the A and C fractions. It disappeared when the A fraction was repurified as described under analyses (*see* previous section).

Potency of Fractions

Tests on guinea pigs: The results of skin tests on guinea pigs made with comparable doses of the fractions tested (0.005 mg. of protein as calculated from their nitrogen contents) are given in table 6. In the table the average dimensions of the simultaneous reactions in the three sets of experiments are given for the twenty-four-hour and the forty-eight-hour readings. The 107 (A) and 108 (A) fractions were the most potent and even more potent than PPD-S. The 107 (C) and 108 (C) fractions gave the smallest reactions. These results confirm many previous findings with A and C proteins (19). The 107 and 108 glycoprotein fractions gave reactions intermediate between A and C.

Tests on humans: The comparative skin tests on the guinea pigs were definite and reproducible from experiment to experiment, even in small groups of animals, and, therefore, a similar comparison was attempted in humans. Small series of tests were made with comparable doses (based on the nitrogen values) of several different preparations of each type of protein, each in comparison with the standard PPD-S. The groups on the whole were small, but it was considered of more value at present to make a preliminary survey of the possible reproducibility of results from many preparations than to make large surveys with a few. Large surveys are in the future program. It was shown previously (3) that, when two substances were compared in the manner herein described, a mean difference of more than 10 per cent in the sizes of the reactions to the two substances indicated a statistically significant difference in their potencies.

As may be seen in table 7, the different preparations of the A or A+ glycoprotein type of protein were nearly equal to or less potent than the PPD-S in highly hypersensitive persons who reacted at the 0.00002 mg. dosage level. Indeed, in some instances the preparations failed to elicit reactions which were

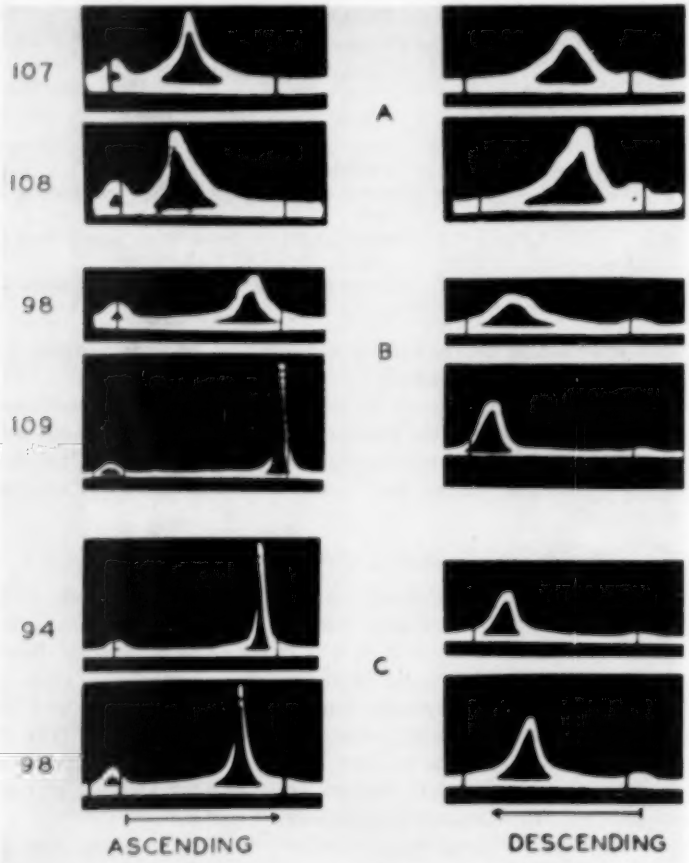

FIG. 1. Electrophoretic diagrams of two each of the purest A, B, and C proteins. All were run in phosphate buffer, pH 7.6, $\mu = 0.1$, at a potential gradient of 9 to 10 volts. Fraction 107 A ran for 79 minutes and had a mobility of -4.1; 108 A, 84 minutes, mobility -3.9; 98 B, 90 minutes, mobilities -6.4 and -7.4; 109 B, 115 minutes, mobility -6.5; 94 C, 74 minutes, -6.3; 98 C, 90 minutes, -6.4.

detected by the PPD-S. (Such reactions are marked in table 7 in parentheses.) The C type of protein in all cases was less potent, especially in two preparations. In many cases, reactions were produced by PPD-S but were not elicited by the C type of protein. On the other hand, four of seven preparations of the B type tuberculin protein or the bacillary protein gave larger reactions than the PPD-S.

In persons with lower hypersensitivity, i.e., those reacting only at the 0.005 mg. dosage level, most preparations of all types were more potent in comparison with PPD-S than at the lower dosage level, and some were considerably more potent. The C proteins again were the least potent.

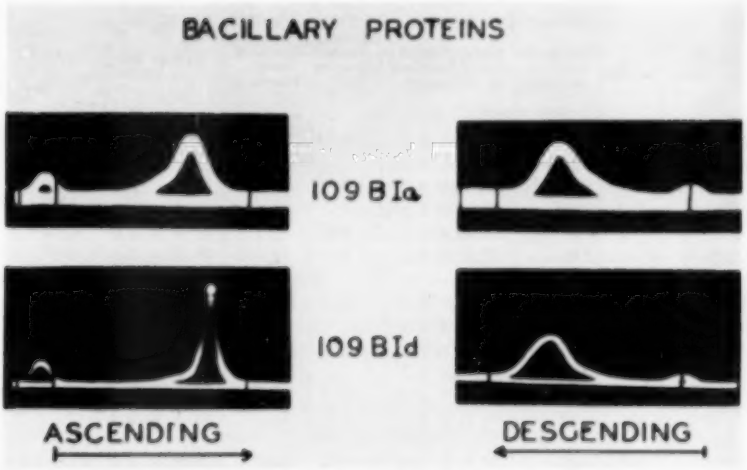

FIG. 2. Electrophoretic diagrams of two urea-extracted bacillary proteins run in phosphate buffer, pH 7.6, $\mu = 0.1$, at a potential grade of 9 to 10 volts. Fraction 109 B Ia ran for 95 minutes and had a mobility of -6.9; and 109 B Id ran for 90 minutes and had a mobility of -6.5.

TABLE 6

POTENCY OF FRACTIONS FOR GUINEA PIGS

Comparative Skin Tests in Guinea Pigs with 107 and 108 Fractions

Experiment	Number of Guinea Pigs Tested	Fraction Tested	Lot Number	Average Dimensions in Millimeters of Reactions with 0.005 Mg. Doses at	
				24 Hours	48 Hours
I	6	PPD-S		17.0 × 2.3	17.6 × 2.5
		A	107	20.1 × 2.8	21.5 × 2.6
		GP	107	19.5 × 2.5	17.1 × 2.3
		C	107	13.9 × 1.8	15.0 × 1.8
II	3	PPD-S		17.7 × 2.7	17.2 × 3.0
		A	108	21.7 × 3.0	20.0 × 3.0
		GP	108	10.5 × 2.0	3.0 × trace
		C	108	9.7 × 2.0	13.5 × 2.0
III	6	PPD-S		16.3 × 2.5	19.5 × 2.5
		A	107	20.8 × 2.3	20.2 × 2.0
		GP	107	16.3 × 2.0	17.3 × 2.0
		C	107	15.3 × 2.0	16.7 × 2.2
		A	108	22.7 × 2.3	22.8 × 2.4
		GP	108	15.7 × 1.8	12.6 × 1.6
		C	108	12.9 × 1.6	11.7 × 1.4

GP = glycoprotein.

TABLE 7

Potency of Fractions for Humans

Comparative Skin Tests in Human Beings

Type of Protein Tested	Lot Number	0.00002 Mg. Dose			0.005 Mg. Dose		
		Number of Positive Reactions	Average Dimensions of Reactions in Millimeters	Difference in Average Diameters from PPD-S	Number of Positive Reactions	Average Dimensions in Millimeters	Difference of Average Diameters from PPD-S
A	100	29	22.8 × 2.2	−0.6	10	19.7 × 1.9	+1.1
PPD-S		29	23.4 × 2.2		10	18.6 × 1.5	
A	107	24	25.7 × 2.2	−2.0	10	14.8 × 1.6	−2.2
PPD-S		24	27.7 × 2.1		10	17.0 × 1.8	
A	108	41 (4)*	22.1 × 2.2	−5.6	12 (1)	19.9 × 2.0	0
PPD-S		41	27.7 × 2.4		12	19.9 × 2.0	
A+GP	109	41	23.9 × 2.3	−0.3	12	21.9 × 2.3(1 M)	−1.8
PPD-S		41	24.2 × 2.4		12	23.7 × 2.6	
A+GP	96B	103	18.3 × 2.1	−6.0	238	19.1 × 1.6	+4.6
PPD-S		103	24.3 × 2.5		238	14.5 × 1.3	
A+GP	98	19	24.7 × 2.5	−0.1	13	23.9 × 2.4	+2.5
PPD-S		19	24.8 × 2.4		13	21.4 × 2.2	
GP	100	135	27.0 × 2.6	−0.3	347	26.4 × 2.0	+7.7
PPD-S		135	27.3 × 2.6		347	18.7 × 1.6	
GP	109	45 (3)	23.3 × 2.1	−2.4	6 (2)	21.3 × 1.7	−4.1
PPD-S		45	25.7 × 2.4		6	25.4 × 2.2	
B	94	24	33.2 × 2.8	+3.6	10	19.2 × 2.1	+1.5
PPD-S		24	29.6 × 2.8		10	17.7 × 2.1	
B	97	93	29.3 × 2.6	+1.3	101	22.6 × 2.0	+4.3
PPD-S		93 (4)	28.0 × 2.6		101 (5)	18.3 × 1.9	
B	98	10	28.3 × 2.8	0	7	17.9 × 1.8	+0.6
PPD-S		10	28.3 × 2.7		7	17.3 × 1.8	
B	109	34 (1)	19.2 × 2.1	−2.5	10	17.1 × 1.8	−3.1
PPD-S		34	21.4 × 2.2		10 (1)	20.2 × 2.0	
C	92	10 (2)	13.7 × 2.0	−11.8	10 (1)	17.0 × 2.2	−2.7
PPD-S		10	25.5 × 2.3		10	19.7 × 2.3	
C	94	22	24.8 × 2.4	−2.8	10	17.9 × 1.9	0
PPD-S		22	27.6 × 2.4		10	17.9 × 1.9	
C	95	44 (11)	19.5 × 2.0	−8.7	5 (9)	21.5 × 2.3	−4.4
PPD-S		44	28.2 × 2.5		5	25.9 × 2.3	
C	98	84	30.5 × 2.0	−12.4	126 (3)	20.2 × 1.9	−0.6
PPD-S		84	32.9 × 2.7		126	20.8 × 1.9	
C	100	16 (2)	16.7 × 2.1	−7.6	7 (2)	16.0 × 1.7	−0.4
PPD-S		16	24.3 × 2.5		7	16.4 × 1.7	
Bacillary Proteins							
BP	101	27	20.9 × 2.2	−2.8	18	23.8 × 2.2	+5.7
PPD-S		27	23.7 × 2.3		18	19.5 × 2.1	
BPII	109	51	32.4 × 2.4	+2.7	7 (1)	29.6 × 2.1 (1M)	+8.6
PPD-S		51	29.7 × 2.4		7	21.0 × 1.7	
BPId	109	34	30.7 × 2.4	+1.8		Not tested	
PPD-S		34 (3)	28.9 × 2.3				

* The numbers in parentheses indicate the number of reactions missed by one of the products tested. The reactions to the other fraction were not included in the average dimensions given for it.

GP = Glycoprotein.

BP = Bacillary protein.

TABLE 8

RELATIVE POTENCIES OF FRACTIONS IN HUMANS

Differences in Average Sizes of Reactions of the Fractions Relative to PPD-S, in Millimeters

Fraction	Dosage Level	
	0.00002 Mg.	0.005 Mg.
A or A + Glycoprotein	−6.0 to −0.1 mm.	−2.2 to +4.6 mm.
B	−2.5 to +3.6 mm.	−3.1 to +4.3 mm.
C	−12.4 to −2.8 mm.	−4.4 to 0 mm.
Bacillary fractions	−2.8 to +2.7 mm.	+5.7 to +8.6 mm.

A summary of the ranges of actual differences in the average sizes of reactions of all of the fractions tested in comparison with PPD-S at both dosage levels is presented in table 8.

These results indicate that there are proteins of different relative potencies in raw tuberculin and that some of them, especially those of the C type, are considerably less potent than others.

DISCUSSION

The results in the present study show that there are proteins with different chemical properties in unheated tuberculin. The relative amounts of these proteins vary in different tuberculins even from the same strain of bacillus grown at different times. For example, in previous fractionations it was common to find that the largest percentage of the protein was in the form of the C type, whereas, in the three fractionations given in this paper, the A type of protein predominated. The Wong-Wenzirl medium had been used in the preparation of lots Nos. 107 and 108, and Long's medium was used for lot No. 109. In all of these cases the C fraction was scant, in contrast to earlier preparations, all of which had been made on Long's medium. The conclusion must be reached, therefore, that variable relative amounts of the different proteins are produced from time to time, regardless of the strain, the time of growth, the medium used, or the method of isolation. The necessity thus arises for isolating the different proteins in order to obtain protein fractions with reproducible composition. It is a gratifying fact that such fractions can be isolated by many different methods from any of the raw tuberculins, and they can be identified chemically and especially by means of electrophoresis. Reagents such as ammonium sulfate and alcohol at low temperature and at different ranges of pH have been successfully used for isolation by the present writers, and zinc sulfate was used by Pangborn (20).[9]

It was shown earlier (17) that the amino acid composition of the C protein was different from that of the A type of protein in that it contained more of the

[9] This product proved to be a mixture of A and B proteins and had a potency comparable to similar fractions isolated by the present writers. The high relative potency originally attributed (20, 21) to this fraction was due to the fact that it had been compared with an inferior PPD product

neutral amino acids, leucine, phenylalanine, and valine, and less glutamic acid plus alanine, glycine, arginine, and lysine. The presence of an amino acid not commonly found in nature, α-ϵ-diaminopimelic acid, but here noted in the glycoprotein fractions and to a lesser extent in the A type of protein, may explain the previously reported high content of lysine in these proteins, since the method then used for determining the amino acids (17) would not distinguish between these two substances. The C type of protein contained little, if any, of this amino acid. It is interesting that this amino acid was identified in the lipopolysaccharide fraction obtained from the bacillary bodies by Asselineau, Choucroun, and Lederer (22).

In other ways also the C type of protein appeared to be quite different from the other proteins of tuberculin. It was insoluble at pH 4.0 and had a higher absorption density at its minimum wave length (λ250) in contrast to the A protein, and also had a higher electrophoretic mobility. However, in the latter property only, it resembled the B type of protein.

It is clear from even the small number of skin tests given in this study (table 7) that the protein fractions varied in their tuberculin potencies. The C type of protein was definitely less potent than the A or B proteins. The difference between the A and B proteins was much less marked. Whether or not this is biologically significant can be determined only by large-scale surveys.

The results indicate that an improvement in the method of producing a potent tuberculin would require the preliminary removal of the less potent C type of protein. This can be done easily by precipitation at pH 4.0. Whether it will then be necessary to separate further the A and B type of proteins, instead of using the whole residue fraction, remains for future study to determine. The carbohydrate in this residue fraction could logically be ignored, since it appears to have no influence itself on the potency. In this case, the proper concentration of protein could be assured by calculating dosage on the nitrogen content. It might be advantageous, however, for the sake of purity and constancy of preparation, to remove the carbohydrate and nucleic acid impurities from the A plus B protein by a single precipitation. This could be done with any of a number of different reagents.

The fact that, relative to PPD-S, less difference in potency was found with the A and C type of proteins in the less hypersensitive patients, who react only to the higher dose (0.005 mg.), than in the highly hypersensitive patients, who react to 0.00002 mg. dose, suggests a difference in specificity of the different fractions for these two types of persons. A study on BCG-vaccinated persons may, therefore, be profitable. Such a study is under way.

The relatively high potency of the products isolated from the bacillary bodies, such as lot No. 109 (B II) and lot No. 109 (B Id), suggests that further study of such fractions may be profitable, especially since the fractions isolated from the culture filtrate of the same culture were less potent. Moreover, the method here used for isolating this protein is simple and readily yields considerable quantities of the protein in relatively pure and potent form. Any denaturing effect that the urea may have had on the protein, as, possibly, a change in solubility, apparently has not significantly lowered the biologic potency of the protein. Bacil-

lary proteins have been extracted previously from tubercle bacilli by a number of workers: Heidelberger and Menzel (23), Grönwall (24), Heckly and Watson (25), and by Baldwin, Gilbert, Iland, and Jones (26). The method used in the studies here reported was essentially that of the latter group. Some of these investigators indicated that a very potent product was obtained, but no critical study of the tuberculin potency in humans has so far been made with any of their bacillary products.

SUMMARY

A series of unheated tuberculin filtrates have been fractionated, and yields and chemical analyses of the proteins isolated from them are given. In addition, a highly potent protein was isolated in relatively pure form by urea extraction of the bacillary bodies.

A number of different proteins were identified. These were grouped in classes according to their solubilities and speed in electrophoresis: A, a soluble but "slow" protein; B, a soluble and "fast" protein; C, an insoluble and "fast" protein; and glycoprotein containing a soluble, "slow," and carbohydrate-rich fraction.

Proteins with these properties could be isolated from tuberculins of different strains of tubercle bacilli even when different methods were used. The amounts of the different proteins isolated, however, varied in the different fractionations.

The amino acid, α-ϵ-diaminopimelic acid, which rarely occurs in nature, but which has been found characteristically in certain mycobacteria, was found to be abundant in the glycoprotein fractions and present to a slightly less extent in the A proteins. Less was found in the B protein, PPD-S, and bacillary proteins, and only traces or none at all in the C proteins.

The tuberculin skin potency tests of these fractions indicated that the C type of protein was considerably less potent than the standard PPD-S. The A and B types of protein differed less from PPD-S. The extent of difference varied in patients with different degrees of hypersensitivity.

SUMARIO

Aislamiento, Identificación y Clasificación de las Proteínas de la Tuberculina y del Bacilo Tuberculoso

Una serie de filtrados sin calentar de tuberculina fué fraccionada y se presentan los rendimientos y análisis químicos de las proteínas aisladas de los mismos. Además, se aisló una proteína muy potente en forma relativamente pura por extracción con urea de los cuerpos bacilares.

Se identificaron varias distintas proteínas que se agruparon en clases de acuerdo con su solubilidad y velocidad en la electroforesis: A, proteína soluble, pero "lenta"; B, proteína soluble y "veloz"; C, proteína insoluble y "veloz"; y glucoproteína que contiene una fracción soluble, "lenta" y rica en hidratos de carbono.

Aun usando distintas técnicas, pudieron aislarse proteínas dotadas de esas propiedades de tuberculinas de diversas cepas de bacilos tuberculosos. Sin embargo, variaron las cantidades de las distintas proteínas aisladas en las diversas fraccionaciones.

El ácido amínico, ácido α-ϵ-diaminopimélico, que rara vez se observa en la naturaleza, pero que se ha encontrado típicamente en ciertas micobacterias, se observó en abundancia en las fracciones de glucoproteína y estaba presente en grado algo menor en las proteínas

A. Había menos en la proteína B, en PPD-S y en las proteínas bacilares y apenas había indicios o no existía en las proteínas C.

Las pruebas de la potencia cutánea de la tuberculina de estas fracciones indicaron que la proteína de tipo C era mucho menos potente que el PPD-S estándard. Los tipos A y B de proteína discrepaban menos del PPD-S. La magnitud de la diferencia variaba en enfermos dotados de distintos grados de hipersensibilidad.

RESUME

Isolement, identification et classification des protides de la tuberculine et du bacille tuberculeux

Une série de filtrats de tuberculine non chauffés a été fractionnée; le rendement et l'analyse chimique des protéines isolées des filtrats sont exposés. De plus une protide extrêmement active a été isolée sous une forme relativement pure par extraction de l'urée des corps bacillaires.

Un certain nombre de protéines différentes ont été identifiés. Elles ont été groupées par classes selon leur solubilité et leur vitesse à l'électrophorèse: A, une protéine soluble, mais "lente"; B, une protéine soluble et "rapide"; C, une protéine insoluble et "rapide"; D, une glucoprotéide contenant une fraction soluble "lente" et riche en glucide.

Des protéines possédant ces propriétés pouvaient être isoles de la tuberculine extraite de différentes souches du bacille tuberculeux, même lorsque des procédés différents étaient utilisés. Toutefois, les quantités des différentes protéines isoles varianient dans les différents fractionnements.

L'acide aminé, acide diaminopimélique, qui existe rarement à l'état naturel, mais qui a été spécifiquement découvert dans certaines mycobactéries, a été trouvé en abondance dans les fractions glucoprotéides, et un peu moins abondant dans les protéines A. Il y en avait moins dans les protéines B., PPD-S, et dans les protéines bacillaires; dans les protéines C. il n'y en avait que des traces ou pas du tout.

Les tests cutanés à la tuberculine effectués avec ces fractions indiquaient que la protéine du type C. était beaucoup moins active que le PPD-S standard. Les protéines du type A. et B. différaient moins du PPD-S. L'écart variait chez les différents malades selon le degré de leur allergie.

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ENERGY COST DURING VARIOUS PHYSICAL ACTIVITIES IN CONVALESCING TUBERCULOUS PATIENTS[1]

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Regulation of physical activity is an important principle in the management of chronic disease. It applies permanently to conditions in which the distribution, diffusion, and transport of oxygen are defective, with consequent diminution in work capacity. Regulation of activity may be advisable in convalescence when, after prolonged bed rest, deterioration of cardiovascular, respiratory, neuro-muscular, and other physiologic functions ("deconditioning") temporarily renders the subject unfit for physical activities ordinarily undertaken without difficulty. Grading of stress is still part of the recently enhanced armamentarium for the treatment of tuberculosis, for it is widely accepted that untimely and uncontrolled physical activity may adversely influence the disease. In spite of the widespread use of this principle, however, little information is at hand to define in specific terms its practical application to the patient. All too often, control of physical activity is exercised by the vague admonition to "take it easy," thus erring on the lax side; or by overly severe restriction, which, although erring on the safe side, brings in its train undesirable psychological reactions. It is pertinent, therefore, to inquire into methods whereby physical stress can be selected to fit with some degree of approximation the tolerance of a particular patient.

Stress in this context depends on two mutually related factors: the poor physical fitness of the patient and the increased metabolic demand. Unfortunately, fitness or tolerance to work cannot be readily estimated by the physician, because it is a resultant of many individual physiologic and psychological factors. On the other hand, the approximate demand of any activity can be readily defined in metabolic terms, i.e., the energy expanded to accomplish it. In this sense, work implies two components, intensity and duration. In any environment, duration can readily be controlled by prescription. The intensity factor for many different activities can be established easily by measuring the rate of oxygen consumption during their execution. While, admittedly, the usefulness of such data to estimate stress may be limited, yet, in conjunction with other clinical knowledge, they may contribute to a decision regarding the wisdom of allowing a given activity for a patient. On this basis, one of the present writers working with normal subjects has already carried out determinations of working metabolic rate during various tasks applicable to tuberculous and other pa-

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tients (1). Another investigation has recently added to these data (2). The present paper is concerned with the accumulation of similar observations in actual patients with tuberculosis.

METHOD

Thirty-nine male patients were used, all with tuberculosis which had been noninfectious for four to six months. Most of the patients had received antimicrobial therapy and, in one-third of the group, various degrees of lung resections had been performed. All had been assigned to the rehabilitation ward and were engaging in physical activity for two to four hours daily. The patients' disease was classified on admission according to severity: one as minimal, 23 as moderately advanced, 14 as far advanced, and one as primary complex. Of more importance to this study, a record was kept of the time elapsed from resumption of some ambulatory activity by the patient to the day of the experiment. This was done to ascertain whether rates of working oxygen consumption of persons doing the same tasks varied according to their state of "deconditioning." Of the 39 patients, 29 had assumed some degree of physical activity for three to nine months. Three had had two months or less; 7, ten months or more of physical activity.

The choice of workshop tasks from available facilities was further restricted by a limitation imposed by the nature of the measurements, viz., the approach to a steady state during the determination of oxygen uptake. While this ideal cannot be achieved, it can be approximated if the task consists of one motion pattern or a series repeated many times during the experiment. In this light one can speak, then, of an average oxygen uptake. Available tasks falling within this requirement were few, and four were chosen: *rug-hooking*, *copper-tooling*, *sewing on a pedal-driven machine*, and *chisel-carving*. In addition, these activities were justified by the fact that they are common to most sanatoriums.

Rug-hooking was accomplished by means of an "automatic yarn-feeder," which was repeatedly thrust through the weave of the cloth along the outline of a design. The pace was set at thirty to forty strokes per minute. Copper-tooling consisted of pressing a copper foil with an appropriate tool around a predetermined design; the patient was allowed to set his own optimal rate of work. At the sewing machine, straight lines six inches long were successively stitched five times per minute. At the end of each line the machine was stopped, the material moved to the beginning of the next line, and the cycle repeated. Thus, the work consisted of two motion patterns interrupted by a short pause; but, since at least fifty such cycles were repeated during an experiment, the results tended to approximate an average. These three tasks were all done in a seated position. The fourth task consisted of carving a design in relief out of pine wood with mallet and chisel. Working in a standing position, the patient was allowed to achieve his own pace.

Because in a previous study the oxygen uptake during *showering* was found to be rather high (1), the metabolic cost of this activity was also determined. The temperature of the water was taken frequently during the shower and ranged from 32° to 38°C. To ensure the collection of an adequate amount of expired air (90–120 L.), the subject completed several showers during one session. In this way, although the motions were dissimilar, at least several cycles contributed to an over-all value.

Twenty-five experiments on the four workshop tasks and ten on showering were performed. Because of the turnover on the ward, the population in each of the four workshop tasks was not identical. Oxygen consumption was determined by the open-circuit method, the details of which have been presented in a previous publication (1). The samples of expired air were corrected to standard pressure at both 0° and 37°C. to give *pulmonary minute volume* at both temperatures. The value at 0°C. was used to find oxygen consumption per minute (STPD, dry). Respiratory rates, counted during the collection period (except during showering), along with pulmonary minute volume at 37°C., allowed calculation of average tidal volume at body temperature. Results were expressed in terms of per cent rise of working over basal oxygen uptake, and cc. of oxygen uptake per minute per kg. Oxygen

TABLE 1

OXYGEN CONSUMPTION FOR VARIOUS OCCUPATIONAL THERAPY TASKS, EXPRESSED IN TERMS OF PER CENT RISE OVER BASAL AND CUBIC CENTIMETERS PER MINUTE PER KILOGRAM DURING WORK

Task	Rug-Hooking		Copper-Tooling		Sewing Machine		Chisel-Carving	
	Per Cent	Cc. per Min. per Kg.	Per Cent	Cc. per Min. per Kg.	Per Cent	Cc. per Min. per Kg.	Per Cent	Cc. per Min. per Kg.
Mean	33	4.65	66	5.69	77	6.01	100	7.05
Range	10–56	3.45–6.09	30–105	4.46–7.08	53–114	4.89–7.27	51–191	5.06–8.82
Standard deviation	13	0.74	20	0.76	16	0.66	29	1.05

debt due to showering was also measured: After a suitable volume of expired air had been collected during seated rest and then during showering (subsequent to a three-minute preliminary workout), the subject was immediately seated again and expired air was obtained successively at minute intervals (or less if pulmonary minute ventilation was high) for three minutes to determine the oxygen uptake during the recovery period.

RESULTS

Basal metabolic rate: All subjects showed basal metabolic rates within normal limits, as observed oxygen uptakes agreed within plus-minus 10 per cent of the Dubois standards. Multiple determinations in the same subject also showed good agreement. Of 27 patients on whom two to four determinations of basal metabolic rate were available in each case, in 23 instances the deviation from the mean ranged from 0 to plus-minus 10 per cent; in 18, plus-minus 6 per cent or less. In 4 the deviations fell between plus-minus 12 per cent and plus-minus 16 per cent.

Working oxygen uptake: In table 1 the data on twenty-five experiments in each of the four occupational tasks are listed. As expected, rug-hooking, which involves mainly wrist motion, exhibited only a slight rise (mean, 33 per cent) over basal. This value may be expected to be even slighter in the reclining position, since sitting itself involves a rise over basal of 17 to 25 per cent (3, 4). However, individual variations were large, as evidenced by the values for standard deviation and range. Furthermore, duplicate experiments yielded substantial deviations. In 3 subjects in whom repeat experiments were done within a few days of each other, the following variations were found: 68 and 40 per cent (plus-minus 14); 10 and 35 per cent (plus-minus 13); 38 and 31 per cent (plus-minus 4).

Copper-tooling (table 1) yielded a mean rise in oxygen consumption above basal of 66 per cent, with a spread of 30 to 105 per cent. Deviations in duplicate experiments in 2 subjects were 58 and 30 per cent (plus-minus 14); 77 and 91 per cent (plus-minus 7). In 5 healthy occupational therapists performing the same task, one of the present writers found a mean rise of 41 per cent, with a range of 27 to 50 per cent (1). Apparently, in subjects familiar with and, perhaps, interested in a task, economy of work is of a higher and less variable order.

The mean rise in working oxygen consumption of 77 per cent for a pedal-driven sewing machine (table 1) was only moderate, compared with that of copper-tooling, even though the lower extremities participate in the former task.

The effect of inertia of the wheel and the small requirements made of the upper extremities in sewing apparently led to comparatively low physiologic demands. Except for one patient, individual deviations from the mean were moderate: standard deviation, 16; spread, 53 to 114 per cent (table 1). In one duplicate observation the difference was similar to those of rug-hooking and copper-tooling: 63 and 84 per cent (plus-minus 11).

Of the workshop tasks, the highest metabolic cost was related to chisel-carving, during which fairly vigorous movements of the upper extremities were made in the standing position. The mean rise of 100 per cent is also associated with a standard deviation of 29 and an enormous range of 51 to 191 per cent (table 1). If one aberrant value of 191 per cent is eliminated, a more nearly accurate picture may be obtained. This correction is justified because the patient, not only was tense, but worked harder, having chiseled the wood to a deeper level than any of the others. The data then become: mean rise, 96 per cent; range, 51 to 149 per cent; standard deviation, 22.

Although the mean values of oxygen uptake in the four tasks form a progression series corresponding to the mass of active muscle, adjacent members of the series overlap to a considerable degree. The statistical mean offers only a rough prediction of the actual metabolic cost in an individual.

The wide individual scatter about the mean was also apparent in the showering experiments (table 2). The per cent rise in oxygen uptake over basal ranged from 184 to 381 per cent. These values substantiate previous findings on 5 normal subjects who exhibited rises of 242 to 377 per cent above basal (1). In spite of the wide deviations, the fact remains that this activity was accompanied by a high observed oxygen uptake. Even then the results did not represent the full oxygen requirements for two reasons: maximal oxygen uptake per minute associated with a steady state had probably not yet been reached during the actual recording period, since the preliminary warm-up lasted only three minutes;

TABLE 2

OXYGEN CONSUMPTION AND DEBT IN SHOWERING

Subject	Oxygen Consumption Cc. per Min.	Per Cent Rise Over Basal	Cc. per Kg. per Min.	Oxygen Debt Cc.-Min.
J. R.	862	349	15.28	1.5 L.—3 min.
E. L.	986	381	14.18	1.3 L.—2.75 min.
J. D.	1,008	285	13.95	1.1 L.—4 min.
G. G.	813	297	12.29	0.8 L.—4 min.
L. R.	834	278	11.99	1.2 L.—4 min.
J. K.	604	230	9.99	0.8 L.—4 min.
G. X.	713	244	9.95	0.9 L.—3.5 min.
S. M.	723	189	9.42	0.7 L.—4 min.
G. S.	892	202	9.12	1.0 L.—3 min.
P. B.	590	184	8.10	0.4 L.—4 min.
Mean	—	264	11.43	—

second, a measurable oxygen debt, discharged in three to four minutes, was found as a result of this activity (table 2).

From table 2 the magnitude of the oxygen debt from showering appears to be roughly proportional to the order of rise in metabolism. An even better correspondence is evident when related to working oxygen consumption in cubic centimeters per kilogram per minute. However, this size of debt probably does not pertain under usual conditions. It is to be remembered that for experimental purposes the total period of showering lasted approximately eight minutes, and this prolongation might have favored the accumulation of any oxygen debt considerably above that found under ordinary circumstances. Nonetheless, the intensity of work associated with showering, no matter how short the duration, is much higher than any of the occupational tasks tested, and even then the observed oxygen uptakes represent an estimate which errs on the low side for the two reasons mentioned.

Respiratory data: In convalescing tuberculous patients, large increments in depth of respiration may be inadvisable because of adverse effects upon bronchial ulceration and healing cavities. In a limited number of experiments presented in a previous communication, increments in tidal volume above 20 per cent of basal were found when the rise in working oxygen uptake exceeded the basal metabolic rate by 60 per cent. This relationship was not verified in these one hundred experiments on four occupational therapy tasks. In fact, a correlation between increments of working tidal volume and oxygen uptake could be discerned neither in individual members of each group nor in group averages. Absence of parallel between tidal volume and intensity of work is not surprising, as a reciprocal relationship is often found between tidal volume and respiratory rate. Hence, a much better index of ventilation is the pulmonary minute ventilation.

In each work group the degree of rise in pulmonary minute ventilation could not be predicted from the change in working oxygen uptake. Correlation coefficients and probabilities of significance were found to be of a low order except for copper-tooling, in which values were 0.55 and less than 1 per cent, respectively. These calculations merely indicated a great variability of respiration under the experimental conditions, although it is well-known that within certain limits a change in metabolism produces a proportionate change in ventilation. Indeed, such a relationship could readily be demonstrated when group averages in the five tasks were ranged in ascending order of working metabolism: per cent rise of oxygen uptake—33, 66, 77, 96,[4] 264; per cent rise of pulmonary minute ventilation—35, 70, 66, 87, 204. It appears from these experiments, therefore, that undue mechanical stress on the lungs in workshop tasks is not consistent, particularly since tidal volume does not usually follow the magnitude of change in metabolism. Only showering, with a mean rise of 204 per cent in pulmonary minute ventilation, appears to deserve some concern in this connection.

Influence of modified bed rest: Deterioration in physiologic functions are recog-

[4] Corrected according to explanation presented above with respect to wood-carving.

nized to set in during a period of prolonged bed rest (5). In convalescence, the evidence of such deterioration during work is much more difficult to obtain (6). Therefore, it was thought interesting to ascertain whether the length of the period from strict bed rest to the day of the experiment was related to the magnitude of rise in oxygen uptake in a given task. Such a correlation has not been found in patients following a short acute illness (6). Referring to the data on showering (table 2), patient P.B. exhibited the lowest response (184 per cent), although the time from strict bed rest was one month; and patient E. L., with three months elapsed time, represented the highest rise observed (381 per cent). Conversely, patient G. S., who had been on modified bed rest for twelve months, showed a relatively low oxygen uptake (202 per cent); and patient L. R., on eleven months' physical activity, a large oxygen uptake (278 per cent).

If differences due to "deconditioning" could be detected by the method used, a stressful activity such as showering would be more likely to bring them to light. The other activities tested also failed to reveal any correlation between the order of rise in oxygen uptake and the length of time from strict bed rest. There is the possibility, however, that, if such a relationship existed, it might be discernible only within a very short time after resumption of physical activity.

DISCUSSION

The rather large individual variations within each activity tested in the present study are inherent in biometrics of this kind. Aptitude and training may determine to a certain extent the rhythm, rate, and intensity of muscular movement. When these two characteristics are present to a small degree, waste motion is not only favored, but more variable. Thus, in a previous investigation in which occupational therapists worked on copper-tooling, the difference between maximum and minimum was only approximately one-half that found here. In addition, the increment in energy expended in terms of oxygen consumption was lower, as mentioned above.

Another factor influencing variation in oxygen uptake appears to be the nature of the task itself. The progressive increase in metabolic work as the subject goes from rug-hooking to showering, in general, is associated with increasing magnitude of variation from the mean. However, tasks such as rug-hooking and sewing, where rate of work was controlled, show smaller deviations. Operations without precise regulation and involving larger muscle masses may favor wider variations in motion patterns and, consequently, in metabolic work. This is actually another way of saying that training is important and results in economy of work. Nonetheless, the large variations in a given task do not vitiate the study, because it was of interest to assess the metabolic expenditure of patients in realistic situations and not under ideal conditions.

In addition to individual differences, the limitations of methodology also make for variability of results. Measurement of oxygen uptake implies the achievement of a steady state. Such an ideal was only approximately attained in the three-minute preliminary workout prior to the actual collection of expired air. During the collection period it is likely that there existed in the various

subjects differing degrees of deviation below the maximum steady oxygen uptake possible for a particular activity. Another uncontrollable metabolic event arises from the fact that, if oxygen debts had accrued, particularly in the tasks involving increments of 100 per cent or more, then the determined oxygen uptake did not express the total energy expenditure.

These two physiologic characteristics, steady state and oxygen debt, represent inherent limitations erring on the low side; but the derived data fix at least a minimal level of energy expenditure. On the other hand, lack of a good degree of training in a task may have tended to influence the results in the opposite direction. Deterioration of physiologic functions due to previous bed rest did not appear to affect the results obtained.

Due to such spreads, the four workshop tasks, when considered individually, overlap to a considerable extent. For example, the lower limits of machine-sewing and chisel-carving include the upper limit of copper-tooling. For this reason, even if it were advisable to do so, a table relating various tasks with the order of oxygen uptake cannot be used as an "energy pharmocopeia." The purpose of these data is only meant to afford some idea of the degree of stress imposed by various tasks and to aid the clinician in more realistically prescribing activities in these terms. Specifically, it would seem contradictory to allow a patient to shower and yet deny him some simple operation in bed, such as rug-hooking. In line with this concept, it is emphasized that the data presented are related to *intensity* of physiologic effort rather than the total amount of work done. Showering itself may take two or three minutes and will contribute an insignificant amount to the total daily expenditure; yet the relatively high intensity of physiologic stress engendered even for a short time may exceed a certain critical level above which injury may ensue. Incidentally, these findings can be applied as well to patients with limited cardiac and pulmonary reserve in whom the work capacity below the limit of dyspnea is known in terms of oxygen uptake.

Variable as the results are, such studies are still superior to assessment of severity of work by inspection. For example, carving with chisel and mallet in the standing position gave an average increment of energy expenditure of 100 per cent. In twelve occupational therapists who were chisel-carving in a seated position without benefit of mallet, the average rise was 122 per cent (1). Doubtless, the latter value would have been even higher if derived from patients. Yet, by kinesiological deduction, as usually done, one would probably have ascribed higher energy expenditure to the former type of task. Apparently the high degree of tension developed in muscles in order to direct the instrument deep into the wood and yet maintain the necessary control without a mallet makes even a larger metabolic demand than the force developed in manipulating one, even though a larger muscle mass may be involved. Pure kinesiological observation can take into account merely the general musculature used and not the intensity of muscular contraction. Only oxygen consumption measurements are capable of grading the severity of physiologic work.

This statement is also borne out by the results obtained during showering.

They are a source of surprise to many because they are higher than expected purely from inspection. However, a moment's thought will reveal that all the musculature of trunk and limbs contribute to the work. Moreover, the fact that magnitude of energy expenditure is considerable is indicated by the contraction of rather large oxygen debts. Indeed, as has already been discussed in the foregoing, the values derived probably represent an underestimate of the energy cost of showering. Incidentally, from the point of view of work physiology, it is of interest that an activity requiring an oxygen uptake of 600 to 1,000 cc. is yet associated with a measurable oxygen debt. Evidently, even moderate intensities of physiologic demand require an anaerobic settlement.

That the temperature of the water can play a significant part in the observed rise of oxygen uptake in showering is controverted by the following facts: (*a*) metabolism rises only 7 per cent with 1°F. rise of body temperature; (*b*) the body temperature probably did not rise to the upper limit of the water temperature (38°C.); (*c*) even if it did, only 14 per cent could be attributed to the final results of 184 to 381 per cent.

No conclusions can be reached relative to the mechanical effect of increased ventilation upon the lungs, as the results were too variable. At any rate, it may be presumed that increased oxygen uptake is associated generally with increased respiratory activity.

In the enthusiasm engendered by new surgical methods and antimicrobial drugs, the physician tends to drive hard for laboratory negativity with little attention to physical stress, deemed so important only recently. It is the writers' opinion that supervision of physical stress is still necessary in the management of the tuberculous patient. Admittedly, no tangible evidence is yet available describing the adverse influence of exertion upon the healing or stabilized focus. Nevertheless, in the past fifty years follow-up studies have allowed the observer to suspect uncontrolled physical activity in the mechanism of recurrence; and, at present, there is no body of knowledge to justify rejection of this concept. Indeed, precisely because of this clinical suspicion, it is sounder practice to honor than to violate the doubt and to continue to use caution. For this reason, any method is desirable which contributes to the control of stress by more accurate dosage of physical activity.

SUMMARY

Restriction of physical stress plays a role in managing chronic disease, yet practical methods for matching this to the patient's tolerance are not available. One useful criterion may be the metabolic intensity of a given physical activity. In this light, several occupational therapy tasks and showering have been assessed in 39 convalescing tuberculous patients in terms of oxygen uptake.

Although individual variations in a given activity were large, a general idea of the magnitude of oxygen uptake has been afforded in rug-hooking, copper-tooling, sewing on a pedal-driven machine, chisel-carving with mallet, and showering. The mean values progress in the order stated. With the last-named activity, taking a shower, a measurable oxygen debt has been found.

Effects of work on pulmonary minute volume and tidal volume were irregular and showed no correlation among subjects in a given task. However, the average increments of oxygen uptake for the five activities paralleled those of pulmonary minute volume.

SUMARIO

Costo de la Energía Durante Varias Actividades Físicas en los Tuberculosos Convalecientes

La limitación del esfuerzo físico desempeña un papel en la asistencia de las enfermedades crónicas, y sin embargo, no se cuenta con métodos prácticos para ajustarla a la tolerancia del enfermo. Una pauta útil tal vez sea la intensidad metabólica de cierta actividad física dada. Desde ese punto de vista, se han justipreciado varias tareas de ergoterapia y baños de ducha en 39 tuberculosos convalecientes en términos de toma de oxígeno.

Aunque fueron considerables las variaciones individuales en una tarea dada, se obtuvo una idea general de la magnitud de la toma de oxígeno en el tejido de alfombrillas, labrado en cobre, costura en máquinas de pedal, cincelado con mazo y baños de ducha. Los valores medios suben en el orden expuesto. En el último renglón se observó un déficit mensurable de oxígeno.

Los efectos del trabajo sobre el volumen pulmonar por minuto y el volumen corriente fueron irregulares, sin mostrar correlación entre distintos individuos en una tarea dada. Sin embargo, los incrementos medios de la toma de oxígeno para las cinco tareas fueron paralelos a los del volumen pulmonar por minuto.

RESUME

Dépense d'énergie au cours de diverses activités physiques chez les tuberculeux convalescents

La restriction de la fatigue physique joue un rôle dans le traitement des affections chroniques, toutefois, les méthodes permettant de les équilibrer par rapport à la tolérance du malade font défaut. Un critère utile pourrait être l'intensité du métabolisme dans une activité physique donnée. Dans ce but diverses formes d'exercices de rééducation et prenant une douche ont été prescrits et mesurés en termes de consommation d'oxygène chez 39 tuberculeux, convalescents.

Malgré l'existence d'importantes variations individuelles pour un exercice donné, une idée générale de la valeur de la consommation d'oxygène a été obtenue dans les travaux suivants: tissage de tapis, martelage du cuivre, travail à la machine à coudre à pédale, sculpture au ciseau à l'aide du marteau et prendre une douche. Les valeurs moyennes augmentent graduellement dans l'ordre indiqué. Dans prenant une douche on obtient un déficit d'oxygène mesurable.

Les effets du travail sur le volume de ventilation par minute et sur le volume de l'air courant étaient irréguliers et sans corrélation lorsqu'ils ont été étudiés chez des individus exécutant un travail donné. Toutefois les incréments moyens pour la consommation d'oxygène, dans ces cinq formes d'activité, étaient parallèles au volume de la ventilation par minute.

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STUDIES ON THE STABILITY OF ISONIAZID[1]

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INTRODUCTION

Isoniazid is generally considered to be a relatively stable compound. For example, when solutions of isoniazid in liquid culture media appropriate for tubercle bacilli are sterilized in the autoclave, no detectable diminution in the antibacterial activity of isoniazid results. Recent observations indicate, however, that the stability of isoniazid is intimately related to the chemical composition of the environment and that isoniazid may be destroyed on exposure to heat or to oxidizing agents under certain conditions. This communication presents the results of experiments dealing with factors found to influence the the stability of isoniazid *in vitro*.

MATERIALS AND METHODS

Colorimetric test: Short has recently described a colorimetric method for the determination of isoniazid in serum (1). After protein precipitation and addition of sodium hydroxide, a color is obtained employing the reagent 1,2 naphthoquinone-4-sulfonic acid. A modification of Short's method, developed in this laboratory, has proved satisfactory for the determination of isoniazid in protein-free solutions. Ten milliliters of the sample were pipetted into a 25 by 150 mm. Pyrex tube. Then, 4 ml. of distilled water, 2 ml. of 0.1 per cent aqueous solution of 1,2-naphthoquinone-4-sulfonic acid, and 4 ml. of N/2 sodium phosphate were added in that order. The tubes were allowed to stand in the dark for one-half hour to permit development of the color and then read in a Coleman Junior spectrophotometer at 455 mμ, using appropriate blanks. As the concentration of isoniazid was not directly proportional to the optical density, values were read from a calibration curve, prepared from solutions of known concentrations of isoniazid. Results were reported to the nearest 0.5 γ per ml.

Liquid medium: The liquid "base medium" used was that of Dubos and Middlebrook (2) and had the following composition:

$Na_2HPO_4 \cdot 12\ H_2O$	0.63 per cent
KH_2PO_4	0.1 per cent
Asparagine	0.2 per cent
Enzymatic hydrolysate of casein (N-Z Amine, Type B, Sheffield)	0.05 per cent
Ferric ammonium citrate	0.005 per cent
$CaCl_2$	0.00005 per cent
$MgSO_4 \cdot 7\ H_2O$	0.001 per cent
$ZnSO_4 \cdot 7\ H_2O$	0.00001 per cent
$CuSO_4 \cdot 7H_2O$	0.00001 per cent

These ingredients were dissolved in distilled water, the pH was adjusted to 6.5 to 6.8, and bovine albumin (fraction V), glucose, and Tween® 80 were added as indicated in the particular experiments. The distilled water used in various tests was obtained from a Barnstead steam still equipped with blocked tin liner and taps.

Autoclaving: Autoclaving was for fifteen minutes at 15 pounds of pressure (121°C.).

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RESULTS

While employing the colorimetric determination for isoniazid described above, it was observed, by chance, that isoniazid was apparently destroyed when autoclaved in aqueous solution. Because of this observation, further investigation was undertaken.

Influence of pH on the Heat Stability of Isoniazid Solutions

The initial studies were concerned with the effect of variation in pH on the heat stability of isoniazid.

Three tubes were prepared, each containing 1 ml. of isoniazid solution (50 γ per ml.) and 7 ml. of distilled water. One ml. of 4 per cent KH_2PO_4 and 1 ml. of 3 per cent $Na_2HPO_4 \cdot 12\ H_2O$ were added as follows: To one, the monobasic phosphate before autoclaving, the dibasic after (solution autoclaved at pH 4.8); to a second, both phosphate solutions added before autoclaving (solution autoclaved at pH 6.5); and to a third, the dibasic phosphate before autoclaving, the monobasic after (solution autoclaved at pH 8.8). All solutions had a final pH of 6.5 with an initial concentration of 5 γ of isoniazid per ml.

The results of colorimetric analyses, given in table 1, were as follows: In the solution autoclaved at pH 8.8, 90 per cent of the isoniazid initially present had disappeared; when the isoniazid was autoclaved in solution at pH 6.5, an 80 per cent loss occurred; and in the solution autoclaved at pH 4.8, the isoniazid appeared to be almost completely stable. Solutions prepared as in the above experiment were also examined for residual isoniazid by microbiologic assay.

As described above, serial dilutions of each of the three solutions of isoniazid, autoclaved at pH 4.8, 6.5, and 8.8, respectively, were made in aluminum capped tubes. To 1 ml. volumes of these dilutions were added 4 ml. of "base medium" containing 0.5 per cent albumin, 0.5 per cent glucose, and 0.05 per cent Tween® 80. The tubes were then inoculated with a 10^{-3} final concentration of a fully grown Tween®-albumin culture of BCG-Phipps (bovine attenuated strain of *M. tuberculosis*) and incubated at 38°C. for a period of seven days.

As is shown in table 2, the values for residual isoniazid concentrations after autoclaving, as determined microbiologically, were well correlated with those obtained by the colorimetric method. The isoniazid solution autoclaved at an alkaline pH showed at least a tenfold decrease in antibacterial activity, and

TABLE 1

HEAT STABILITY OF ISONIAZID AT VARIOUS RANGES OF pH

Solutions of Isoniazid in Phosphate Buffer Autoclaved at pH	Concentration of Isoniazid After Autoclaving*
	γ *per ml.*
4.8	4.5
6.5	1.0
8.8	0.5
(Unautoclaved control)	5.0

* All solutions initially contained 5 γ of isoniazid per ml.

TABLE 2

HEAT STABILITY OF ISONIAZID AT VARIOUS RANGES OF pH

Initial Concentration of Isoniazid (Before Autoclaving)	Growth of Tubercle Bacilli		
	Isoniazid Solution Autoclaved at pH 4.8	Isoniazid Solution Autoclaved at pH 6.5	Isoniazid Solution Autoclaved at pH 8.8
γ per ml.			
1.000	0*	0	++++
0.500	0	++	++++
0.100	0	++++	++++
0.050	+++	++++	++++
0.025	++++	++++	++++
0.000	++++	++++	++++

* Amount of growth graded from 0 (no growth) to ++++ (growth equal to that in medium containing no drug).

that autoclaved at neutral pH showed a fivefold decrease when compared with the solution placed in the autoclave at an acid reaction.

It was further found that the alterations in isoniazid which resulted from heating at neutral or alkaline reactions were not reversed by subsequent acidification.

Studies of Certain Substances Which Render Isoniazid Stable to Heat

As stated in the introduction, isoniazid is generally considered to be heat-stable. For example, it may be autoclaved in culture media at pH 6.8 without detectable loss of activity. The stability of isoniazid when autoclaved in culture media, in contrast to its instability in simple aqueous solutions, suggested that some substance or substances present in media might render isoniazid stable to heat. The following experiment was designed to determine if such were true.

Using the liquid medium described under *Materials and Methods* as a guide, three solutions were made as follows: a solution containing the mineral salts in the medium (ferric ammonium citrate, magnesium sulfate, calcium chloride, zinc sulfate, and copper sulfate), a solution of asparagine, and a solution of enzymatic hydrolysate of casein. All solutes were dissolved in phosphate buffer (0.63 per cent $Na_2HPO_4 \cdot 12\ H_2O$ and 0.1 per cent KH_2PO_4) adjusted to pH 6.7, and all concentrations were five times that in the final medium. Thus, one volume of each of the three solutions when added to two volumes of buffer or water resulted in essentially the "base medium." Serial dilutions of isoniazid were made and 0.8 ml. of the above solutions was added to each tube prior to autoclaving, as shown in table 3. The remaining constituents necessary to make the complete base medium were autoclaved separately and added aseptically. Glucose and albumin, final concentrations 0.5 per cent, were included. No Tween® was used. Inoculation was made with a 10^{-3} final concentration of a fully grown Tween®-albumin culture of the BCG-Phipps strain of *M. tuberculosis*. Readings were made after incubation for eleven days at 38°C.

The results, presented in table 3, indicate that isoniazid was completely stable when autoclaved, not only in the complete base medium, but also in

TABLE 3

STABILITY OF ISONIAZID WHEN AUTOCLAVED WITH VARIOUS COMPONENTS OF A STANDARD LIQUID MEDIUM

Initial Concentration of Isoniazid (Before Autoclaving)	Growth of Tubercle Bacilli					
	Substances Below Added to Isoniazid Solutions Before Autoclaving					
	Mineral Salts in Phosphate Buffer*	Asparagine in Phosphate Buffer*	Enzymatic Hydrolysate of Casein in Phosphate Buffer*	Phosphate Buffer* Only	Water	Complete Base Medium
γ per ml.						
1.000	0†	0	0	++++	+	0
0.500	0	0	0	++++	+++	0
0.250	0	0	0	++++	++++	0
0.125	+++	0	0	++++	++++	0
0.063	++++	++++	++++	++++	++++	++++
0.032	++++	++++	++++	++++	++++	++++
0.000	++++	++++	++++	++++	++++	++++

* pH 6.7.

† Amount of growth graded as in previous experiment.

solutions containing either asparagine or enzymatic hydrolysate of casein. Furthermore, the solution of mineral salts partially protected the isoniazid from destruction in the autoclave. It was noted that the mineral salt solution contained a low concentration of citrate (as ferric ammonium citrate). Thus, all groups of substances which stabilized isoniazid included at least one polybasic organic acid.

The next step, therefore, was to study the effect on the stability of isoniazid of several organic acids in addition to those contained in the medium. Colorimetric determination of isoniazid autoclaved in solutions of organic acids generally proved unsatisfactory because of interfering colors produced by some of the organic acids. However, oxalic acid, which gave no interfering color, appeared to stabilize isoniazid. For investigation of the stabilizing effect on isoniazid solution of the other organic acids, biologic assay was employed.

Serial dilutions of isoniazid were made in order to yield, in a volume of 0.5 ml., concentrations ten times higher than those listed in the table. Five-tenths of a milliliter of 0.1 per cent solutions of the substances indicated in table 4 were added to the 0.5 ml. volumes of the isoniazid dilutions, the tubes were autoclaved, and 4 ml. of sterile base medium were then added aseptically. A final concentration of 0.1 per cent albumin was used. Glucose concentration, inoculum, and incubation time were as in the previous experiment.

The results given in the upper portion of table 4 show that citrate as well as the three amino acids used in the experiment, glycine, lysine, and aspartate, prevented destruction of isoniazid in the autoclave, whereas acetate and succinate had no such effect.

The data presented in the bottom portion of table 4 are derived from experiments performed to determine whether the loss of antibacterial activity in the

TABLE 4

STABILITY OF ISONIAZID WHEN AUTOCLAVED IN SOLUTIONS OF VARIOUS ORGANIC ACIDS

Initial Concentration of Isoniazid (Before Autoclaving)	Growth of Tubercle Bacilli							
	Isoniazid Autoclaved in Solutions Containing the Following Substances*							
	Acetate	Succinate	Citrate	Glycine	Lysine	Aspartate	Phosphate Buffer Only	Complete Base Medium
γ per ml.								
3.3	0†	0	0	0	0	0	0	0
1.0	++++	++++	0	0	0	0	++++	0
0.33	++++	++++	0	0	0	0	++++	0
0.1	++++	++++	0	0	0	0	++++	0
0.03	++++	++++	++++	++++	++++	++++	++++	++++
0.00	++++	++++	++++	++++	++++	++++	++++	++++

	Isoniazid Autoclaved in Base Medium Containing the Following*					
	Acetate	Succinate	Citrate	Glycine	Lysine	Aspartate
0.1	0	0	0	0	0	0

* All solutions were made in mixed phosphate buffer at pH 6.7.

† Amount of growth graded and recorded as in the previous tables.

specimens containing acetate or succinate might be due to isoniazid antagonism by these substances rather than isoniazid destruction in the autoclave. The isoniazid used in this part of the experiment was autoclaved in the presence of the complete base medium, a circumstance known to maintain its activity. Neither acetate nor succinate counteracted inhibition of growth by 0.1 γ of isoniazid per ml., and, therefore, neither appeared to be an isoniazid antagonist.

Stabilizing Effect of a Chelating Agent on Isoniazid Solution

The experiments thus indicated that various amino acids or amino acid derivatives as well as citrate and oxalate stabilized isoniazid in the autoclave. All of these substances have the ability to chelate metallic ions, i.e., to form soluble, nonionic complexes with these ions. Therefore, it seemed possible that isoniazid stabilization was related to the property of chelation common to these substances. It was conceivable that some metal ion, present in trace amounts in the distilled water or in the isoniazid preparation itself, was involved in the inactivation taking place in the autoclave, and chelation of such an ion might have prevented the destruction of the isoniazid.

Experiments were designed to test this hypothesis, utilizing ethylenediamine tetra-acetic acid, disodium salt (hereafter referred to as E.D.T.A.), a compound often employed as a chelating agent. The data in table 5 show that a 10^{-5} molar concentration of E.D.T.A. completely protected a 10 γ per ml. solution of isoniazid, buffered to pH 6.7, from destruction on autoclaving. From these observations, it became clear that unbound metal ions, even in trace amounts, had a profound effect on the heat stability of isoniazid in solution.

TABLE 5

EFFECT OF THE CHELATING AGENT ETHYLENEDIAMINE TETRA-ACETIC ACID (E.D.T.A.) ON THE HEAT STABILITY OF ISONIAZID

Concentration of E.D.T.A.	Concentration of Isoniazid After Autoclaving*
	γ per ml.
10^{-5} M	10.0
None	0.5
(Unautoclaved control)	10.0

* All solutions initially contained 10 γ of isoniazid per ml. and phosphate buffer at pH 6.7. Isoniazid concentration was determined colorimetrically.

TABLE 6

EFFECT OF VARIOUS CATIONS ON THE HEAT STABILITY OF ISONIAZID

Cation, 3×10^{-4} M*	Concentration of Isoniazid After Autoclaving†
	γ per ml.
Cu^{++} ($CuSO_4$)	0.0
Zn^{++} ($ZnSO_4$)	8.5
Ca^{++} ($CaCl_2$)	10.0
Mn^{++} ($MnCl_2$)	0.0
Mg^{++} ($MgSO_4$)	10.0
Co^{++} ($CoCl_2$)	6.0
Ni^{++} ($NiCl_2$)	9.0
Fe^{+++} ($FeCl_3$)	3.0
Fe^{++} ($FeSO_4$)	5.0
Control	10.0
Control without E.D.T.A.	0.5
(Unautoclaved control)	10.0

* 10^{-4} M ethylenediamine tetra-acetic acid was included in all solutions unless otherwise indicated.

† All solutions initially contained 10 γ of isoniazid per ml. and phosphate buffer at pH 6.7.

Relationship Between Certain Metal Ions and the Heat Stability of Isoniazid

In the next experiment, an attempt was made to relate the instability of isoniazid to a particular cation or group of cations. Solutions containing various cations, E.D.T.A., and isoniazid, as shown in table 6, were prepared. The E.D.T.A. was included in order to form a complex with the trace ion presumed to be present in the system and presumed to be involved in rendering isoniazid unstable to heat. The concentration of each cation added was three times the maximum binding power of the concentration of E.D.T.A. used. It was realized that the excess of added cation might partially displace the unknown trace ion from the E.D.T.A.

The results are given in table 6. Under the conditions of the experiment, the addition of cupric or of manganous salts led to the complete destruction of the isoniazid originally present. Zinc, cobaltous, nickel, ferric, and ferrous ions re-

sulted in the partial destruction of isoniazid, whereas no isoniazid disappeared in the presence of calcium or magnesium ion. Only cupric and manganous ions led to a greater degree of isoniazid destruction than that in the control containing no E.D.T.A. and no added cations. It is possible that those ions which brought about only partial destruction of isoniazid under these conditions exerted their effect indirectly. For example, they might have displaced from E.D.T.A. the unknown trace cation presumed to be present in the control system.

Stability of Isoniazid Solutions on Exposure to Hydrogen Peroxide

The results thus far had dealt with the destruction of isoniazid when heated in the autoclave. As it seemed likely that this destruction was an oxidative reaction, experiments were done to study the effect on isoniazid of an oxidizing agent in combination with various cations. Hydrogen peroxide was chosen as the oxidizing agent to be used and was employed in 0.01 per cent concentration.

Incubation was for one hour at 37°C. Additions were as indicated in table 7. The colorimetric determination was performed in the usual manner except that, following incubation, 1 ml. of a 0.1 per cent solution of beef liver catalase was added to each of the 10 ml. aliquots to be analyzed in order to decompose any remaining hydrogen peroxide. Three rather than 4 ml. of distilled water were then added.

The data, presented in table 7, show that the effects of 0.01 per cent hydrogen peroxide on isoniazid were similar to those found following autoclaving. First, isoniazid was destroyed in buffered aqueous solutions (pH 6.7) of hydrogen peroxide. Second, this destruction was prevented by E.D.T.A. Third, the presence of cupric or manganous ions markedly enhanced the destructive effect of

TABLE 7

EFFECT OF VARIOUS CATIONS ON THE DESTRUCTION OF ISONIAZID BY HYDROGEN PEROXIDE AT 37°C.

Cation, 3×10^{-3} M	Concentration of Isoniazid After Incubation Without Hydrogen Peroxide*	Concentration of Isoniazid After Incubation with 0.01 Per Cent Hydrogen Peroxide*
	γ per ml.	*γ per ml.*
Cu^{++} ($CuSO_4$)	8.5	0.0
Zn^{++} ($ZnSO_4$)	10.0	8.5
Ca^{++} ($CaCl_2$)	10.0	9.5
Mn^{++} ($MnCl_2$)	0.0	0.0
Mg^{++} ($MgSO_4$)	10.0	9.5
Co^{++} ($CoCl_2$)	9.5	6.5
Ni^{++} ($NiCl_2$)	10.0	8.5
Fe^{+++} ($FeCl_3$)	7.5	0.5
Fe^{++} ($FeSO_4$)	8.5	2.0
Control	10.0	10.0
Control without E.D.T.A.	10.0	5.0

* Incubation was for one hour at 37°C. All solutions initially contained 10 γ of isoniazid per ml., 10^{-4} M ethylenediamine tetra-acetic acid (unless otherwise indicated) and phosphate buffer at pH 6.7.

hydrogen peroxide. The addition of other ions resulted in the disappearance of part of the isoniazid. Only the cupric, manganous, ferric, and ferrous ions, however, effected the destruction of more isoniazid than that which occurred in the control containing no E.D.T.A.

Effect of Certain Cations on the Stability of Isoniazid Dissolved in Water at 37°C.

As may be seen in table 7, in the presence of manganous ion, isoniazid was destroyed on incubation at 37°C. without the addition of hydrogen peroxide. Also, significant disappearance occurred under these conditions in the presence of cupric, ferric, and ferrous ions.

Since isoniazid solutions had been found to become increasingly unstable to heat the more alkaline the environment, it appeared possible that isoniazid stability at incubator temperature might vary similarly, especially in the presence of cupric or manganous ion. In order to investigate such a possibility, isoniazid solutions were prepared at acid, neutral, and alkaline reactions. Phosphate buffer was used, and the cupric and manganous salts and E.D.T.A. were added as indicated in table 8. No oxidizing agent was added in these experiments.

As is shown by the data, in the presence of cupric ion, more isoniazid was destroyed at an alkaline reaction than at the lower ranges of pH. However, such was not true with manganous ion. In the isoniazid solutions containing manganous ion, more isoniazid disappeared at the neutral pH than at the alkaline pH. It was observed that the neutral and alkaline isoniazid-manganous ion solutions which contained no E.D.T.A. were opalescent. The greater destructive effect at a neutral pH than at an alkaline reaction was thus probably due to the increased solubility of manganous ion at more acidic reactions.

Under both neutral and alkaline conditions, the addition of E.D.T.A. seemed to enhance rather than antagonize the destructive effect of manganous ion on isoniazid. The isoniazid-manganous ion solutions which contained E.D.T.A. remained clear at all ranges of pH, thus indicating that chelation of manganous ion by E.D.T.A. prevented precipitation of manganous phosphate or manganous

TABLE 8

EFFECT OF MANGANOUS AND OF CUPRIC IONS ON THE STABILITY OF ISONIAZID AT VARIOUS RANGES OF PH

Added to Isoniazid Solutions	Concentration of Isoniazid After Incubation for One Hour at 37°C.*		
	pH 4.0-4.5	pH 6.5-7.0	pH 9.5-10.0
3×10^{-4} M Mn^{++}	9.5	6.0	8.5
3×10^{-4} M Mn^{++} and 10^{-4} M E.D.T.A.	9.5	1.0	5.5
3×10^{-4} M Cu^{++}	9.5	9.0	4.0
3×10^{-4} M Cu^{++} and 10^{-4} M E.D.T.A.	10.0	9.0	4.0
10^{-4} M E.D.T.A.	10.0	10.0	10.0
No additions	10.0	10.0	10.0

* All solutions initially contained 10 γ of isoniazid per ml. and phosphate buffer.

hydroxide. The increased destruction of isoniazid which took place in those solutions containing E.D.T.A. and manganous ion is difficult to explain, however, for the concentration of free manganous ion would be expected to be lower in the presence than in the absence of E.D.T.A.

DISCUSSION

It has been shown that cupric and manganous ions and, to a lesser extent, ferric and ferrous ions render isoniazid susceptible to destruction under certain conditions, and that this destruction is increased as the temperature is raised or as the solution is made more alkaline. The fact that hydrogen peroxide greatly increases the rate of disappearance of isoniazid in the presence of these ions strongly suggests that the degradation is an oxidative reaction.

It does not seem likely that these ions act primarily by functioning as electron acceptors. For, manganous ion, the lowest oxidation state of manganese, is much more active than ferric ion, and the activity of ferrous ion is only slightly less than that of ferric ion. Albert has reported that isoniazid forms two complexes with cupric ion, a 1 copper-1 isoniazid complex and a 1 copper-2 isoniazid form (3). It is possible, therefore, that the effect of cupric ions in potentiating the breakdown of isoniazid is related to the formation of a copper-isoniazid complex. Such a complex might be more susceptible than free isoniazid to chemical alteration on exposure to certain conditions.

Several features of the isoniazid destruction in the presence of the above-named cations suggest that these ions function similarly to catalysts. The relative slowness of reaction, the variations with temperature and pH, the ion specificity, and the low concentrations of ion required are all typical of catalytic reactions.

The results reported in this communication are well correlated with those of Pansy and associates (4). These investigators found that a decrease in antibacterial activity of isoniazid occurred during incubation in Dubos liquid medium when the pH was above 6.5. They ascribed this effect to the ferric salts in the medium. Also in accord are the results of Boniche and Reif, who recently reported that the destruction of isoniazid in culture media was "catalyzed" by large concentrations (5 mg. per 100 ml. optimal) of manganous chloride (5). The rate of reaction was found to increase with increasing temperature and alkalinity. Middlebrook has likewise observed the disappearance of isoniazid in solutions containing manganese which were allowed to stand in the air (6).

On the other hand, Barry and collaborators have recently reported a decrease of isoniazid activity after incubation in Proskauer and Beck liquid medium and have found that the decrease occurred on incubation of isoniazid in asparagine or magnesium citrate dissolved in phosphate buffer (7). At first glance these findings appear to disagree with those of the present communication. Both asparagine and magnesium citrate have chelating ability similar to that of E.D.T.A. Hence, it is possible that the effect observed by Barry and associates is similar to that seen with manganous ion (*see* table 8) when the addition of a chelating agent increased the destruction of isoniazid in the presence of this cation.

The phenomenon of bacterial resistance to isoniazid may in some way be related to the marked susceptibility of isoniazid to destruction under certain conditions. Middlebrook has shown that many isoniazid-resistant strains of tubercle bacilli are deficient in catalase, an enzyme which catalyzes the breakdown of hydrogen peroxide to molecular oxygen and water (8). Since aerobic microorganisms generally produce hydrogen peroxide in the course of their metabolism, it seems possible that this substance might accumulate at certain loci in the catalase-negative bacterial cell. Hydrogen peroxide would then be available for the oxidative destruction of isoniazid if an appropriate cation were present, and such a mechanism might account for resistance to the antibacterial action of the drug.

Of interest in this regard are the observations of Barclay, Ebert, and Koch-Weser, using isoniazid made radioactive by incorporation of C^{14} (9). Isoniazid was found to be firmly bound to drug-susceptible bacilli, but resistant cells showed little radioactivity after washing. These results might be explained by degradation of isoniazid in the resistant cell and excretion of the products.

The observations presented in this communication demonstrate clearly that the stability of isoniazid varies, depending upon the chemical composition of the environment. In addition to the obvious importance of this fact to the laboratory worker, it is conceivable that phenomena of a similar nature may influence the fate of isoniazid *in vivo*.

SUMMARY

Although isoniazid has been generally considered to be a relatively stable substance, it is in large part destroyed when autoclaved in aqueous solution at neutral or alkaline reactions. Destruction of isoniazid under these conditions may be prevented by addition to the solutions of any of a number of substances having the common property of forming soluble, nonionic complexes with metal ions.

The stabilizing effect of metal binding agents on solutions of isoniazid exposed to heat may be overcome by the addition of slight excesses of certain cations, especially the cupric and manganous ions. Apparently, trace amounts of these or similar cations render isoniazid susceptible to degradation under certain conditions.

Some destruction of isoniazid also takes place on incubation at 37°C. in the presence of free cupric or manganous ions. The rate of disappearance of isoniazid under these conditions is greatly increased on the addition of dilute hydrogen peroxide. It is thus suggested that isoniazid may be destroyed by an oxidative reaction which is catalyzed by certain metal ions.

SUMARIO

Estudios de la Estabilidad de la Isoniacida

Aunque la isoniacida ha sido considerada generalmente como substancia relativamente estable, queda destruída en gran parte cuando se trata al autoclave en solución acuosa a reacciones neutras o alcalinas. En esas condiciones, puede impedirse la destrucción de la isoniacida agregando a las soluciones cualquiera de varias substancias dotadas en común de la propiedad de formar complejos aniónicos solubles con yones metálicos.

Puede contrarrestarse el efecto estabilizador de los fijadores metálicos sobre las soluciones de isoniacida expuestas al calor con la adición de leves excesos de ciertos cationes, y en particular de los yones cúpricos y manganosos. Al parecer, indicios de esos cationes o de otros semejantes susceptibilizan la isoniacida a la degradación en ciertas condiciones.

Además tiene lugar alguna destrucción de la isoniacida al incubarla a 37°C en presencia de yones cúpricos o manganosos libres. En esas circunstancias, la velocidad de la desaparición de la isoniacida aumenta considerablemente al agregar peróxido de hidrógeno diluído. Sugiérese, pues, que la isoniacida puede ser destruída por una reacción oxidante catalizada por ciertos yones metálicos.

RESUME

Etude sur la stabilité de l'isoniazide

Bien que l'I.N.H. soit généralement considéré comme une substance relativement stable, il est en grande partie détruit quand il est chauffé à l'autoclave en solution alcaline ou neutre. La destruction de l'I.N.H. a été prévenue dans ces conditions en introduisant dans la solution l'une des nombreuses substances ayant la propriété commune de former avec l'ion métal des molécules complexes non-ioniques solubles.

L'effet stabilisant des agents fixateurs de métal sur les solutions d'I.N.H. exposées à la chaleur peut être combattu par l'addition d'un léger excès de certains cations, en particulier d'ions manganeux ou cuivriques. Dans certaines conditions des traces de ces cations ou de cations similaires semblent favoriser la dégradation de l'I.N.H.

Un certain degré de destruction de l'I.N.H. se produit pendant l'incubation à 37°C en présence d'ions manganeux ou cuivriques libres. La vitesse de disparition de l'I.N.H. dans ces conditions est considérablement accélérée par l'addition de peroxyde d'hydrogène. Ces faits suggèrent donc que la destruction de l'I.N.H. peut être due à une réaction d'oxydation catalysée par certains ions métalliques.

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THE EFFECT OF STREPTOMYCIN ON MYCOBACTERIUM SMEGMATIS GROWING IN THE PRESENCE OF PUTRESCINE OR AMMONIUM SULFATE[1,2]

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INTRODUCTION

Turbidimetric measurements of the effect of streptomycin on mycobacterial growth have been used in the past by several workers (1–4). Smith (5), also using turbidimetry, reported that streptomycin susceptibility and growth of *Mycobacterium tuberculosis* depended on the nitrogen source supplied in the synthetic culture medium.

In all of these studies streptomycin was added to the medium at one time only, namely, at the time of inoculation with the bacteria. However, the streptomycin susceptibility of a particular organism possibly may not be constant during its entire growth cycle. In addition, the degree of growth inhibition may not depend merely on streptomycin concentration, but rather on the ratio between streptomycin molecules and numbers of bacteria present. The degree of growth inhibition obtained may furthermore depend on the nitrogenous substrate concentration in the medium.

For these reasons experiments were carried out to study the growth-inhibiting effect of various concentrations of streptomycin added at various times during the growth cycle to media containing different concentrations of one of two different nitrogen sources.

Zeller, Owen, and Karlson (6, 7) and Zeller, Van Orden, and Kirchheimer (8) have shown that streptomycin inhibits the diamine oxidase enzyme of *M. smegmatis*. This enzyme oxidatively deaminates diamines with the formation of ammonia. Putrescine (1,4,diaminobutane) was found to be the best substrate for this enzyme.

It seemed of interest to determine if the inhibition of diamine oxidase by streptomycin is correlated with the growth inhibition caused by this substance. Therefore, *M. smegmatis* was grown in the presence of putrescine and ammonium sulfate, respectively. The growth inhibition resulting from the addition of streptomycin under these two conditions could then be compared. If the inhibition of diamine oxidase is related to the inhibition of growth of *M. smegmatis*, one should expect to find a greater degree of inhibition in the putrescine-containing medium.

MATERIALS AND METHODS

The organism employed in these studies was *Mycobacterium smegmatis*, strain 4172-101, obtained originally from Dr. A. G. Karlson (Mayo Foundation, Rochester, Minnesota).

[1] From the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

This streptomycin-susceptible strain has considerable diamine oxidase activity and has been employed by Zeller and his associates (6–8) in their studies on the effect of streptomycin on diamine oxidase.

The following media were employed in this study:

Basal Medium:

Potassium dihydrogen phosphate	0.54 per cent
Disodium hydrogen phosphate	1.43 per cent
Potassium sulfate	0.05 per cent
Magnesium citrate	0.15 per cent
Glucose	0.50 per cent
Tween® 80, 10 per cent	0.20 ml. per 100 ml.

To this medium was added, except when stated otherwise, crystalline bovine serum albumin (Armour) to give a final concentration of 0.2 per cent. Either putrescine or ammonium sulfate was added to the medium to give the final concentrations specified in the respective experiments. The pH was adjusted to 7.0 with sodium hydroxide. The medium

TABLE 1

THE EFFECT OF VARIOUS AMOUNTS OF STREPTOMYCIN WHEN ADDED AT DIFFERENT TIMES ON THE GROWTH OF MYCOBACTERIUM SMEGMATIS IN PUTRESCINE (40 μM) CONTAINING MEDIUM PLUS ALBUMIN (0.2 PER CENT)

Turbidimetric Readings in "Nephelos Units"

Streptomycin Concentration μM	Time of Reading	Controls (No Streptomycin Added)	Streptomycin Added at 0 Hours	Streptomycin Added at 6 Hours	Streptomycin Added at 12 Hours	Streptomycin Added at 24 Hours
	hours					
0.5	0	2	*1*	1	2	2
	6	6	5	*5*	6	7
	12	30	31 (0)*	31 (0)	*32*	32
	24	97	91 (6)	94 (3)	92 (5)	*97*
	36	173	155 (10)	163 (6)	160 (7.5)	160 (7.5)
	48	282	250 (11)	267 (5)	265 (6)	280 (0)
1.0	0	1.5	*1*	1	1	1
	6	9	9	*8*	8	9
	12	30	26 (13)	27 (10)	*30*	30
	24	111	93 (16)	99 (11)	102 (8)	*110*
	36	220	178 (19)	185 (16)	190 (14)	205 (7)
	48	323	266 (18)	260 (19.5)	265 (18)	292 (10)
2.0	0	1.5	*1*	1	1	1
	6	9	6	*9*	10	10
	12	30	9 (70)	28 (7)	*30*	30
	24	111	5 (95)	71 (36)	105 (5)	*110*
	36	220	5 (98)	83 (62)	190 (14)	202 (8)
	48	323	7 (98)	77 (76)	237 (27)	279 (14)
4.0	0	1.5	*1*	1	1.5	1
	6	9	6	*9*	10	10
	12	30	6 (80)	21 (30)	*30*	30
	24	111	3 (97)	18 (84)	85 (23)	*108*
	36	220	3 (99)	17 (92)	107 (51)	152 (31)
	48	323	2 (99)	18 (94)	102 (68)	227 (30)

* The figures in parentheses represent the per cent inhibition of growth.

was sterilized by passing it through an ultra-fine (UF) sintered glass filter. Seven-milliliter amounts of the sterile medium were then dispensed into sterile, aluminum-capped optical cuvettes (Coleman "7-304"). The media in the cuvettes were inoculated with 0.1 ml. of a culture of this organism in medium of the same composition. The inocula were taken from cultures giving readings of approximately 200 nephelos units on the scale of the Coleman model 7 photonephelometer. Since one such unit is equivalent to approximately 500,000 organisms per ml. (9), the bacterial concentration following inoculation of the media in the cuvettes was approximately 1.4 million per ml. Streptomycin was added to the medium from a stock solution of the compound in distilled water, which was then diluted with distilled water so that the addition of 0.1 ml. to the inoculated medium would result in the desired final concentration. Streptomycin sulfate concentrations are reported here in micromolarity units (μM) or micromoles per liter (μM). The concentrations used in these experiments were 4.0, 2.0, 1.0, and 0.5 μM, corresponding to 2.9, 1.46, 0.73, and 0.37 mg. per liter of streptomycin sulfate.

Growth readings were taken with the photonephelometer at the time of inoculation (zero hours) and at six, twelve, twenty-four, thirty-six, and forty-eight hours. All cultures were incubated at 37° C. in a thermostatically controlled air-incubator. Prior to reading, each tube was shaken to resuspend the organisms uniformly. Each recorded reading represents the average of the readings on two replicate cultures.

RESULTS

The effect of streptomycin on *M. smegmatis* growing in medium containing 40 μM of putrescine is shown in table 1 and in the corresponding figure 1. In this experiment, streptomycin, to make concentrations of 0.5, 1, 2, and 4 μM, respectively, was added at zero, six, twelve, and twenty-four hours. The growth readings in "nephelos units" are shown in table 1 for the various concentrations and times of addition of streptomycin and for the growth in the absence of streptomycin. The figures in parentheses indicate the percentage of growth inhibition. The curves plotted from the growth readings on an arithmetic scale are shown in figure 1. The shaded areas between the control and experimental curves correspond to the inhibition of growth resulting from the addition of streptomycin at the particular time and in the particular concentration.

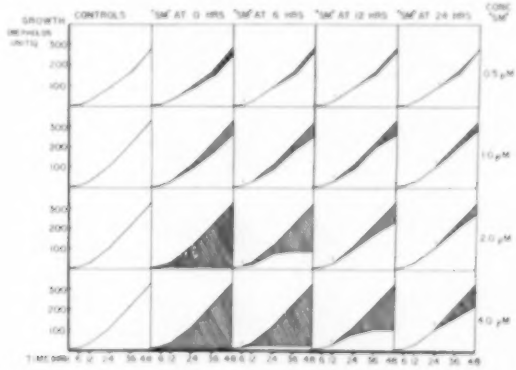

FIG. 1. Graphic representation of the effect of various amounts of streptomycin when added at different times on the growth of *Mycobacterium smegmatis* in putrescine (40 μM)-containing medium, with albumin (0.2 per cent). "SM" = streptomycin.

These results show that streptomycin is a less effective growth inhibitor, not only at lowered concentrations, but also when it is added at a later time.

Reduction of the putrescine concentration in the medium has the effect demonstrated in table 2 and figure 2. Media containing 10, 1, and 0.1 μM of putrescine

TABLE 2

THE EFFECT OF STREPTOMYCIN (1.0 AND 2.0 μM) WHEN ADDED AT DIFFERENT TIMES ON THE GROWTH OF MYCOBACTERIUM SMEGMATIS IN A MEDIUM CONTAINING DIFFERENT CONCENTRATIONS OF PUTRESCINE PLUS ALBUMIN (0.2 PER CENT)

Turbidimetric Readings in "Nephelos Units"

Putrescine Concentration in μM	Time of Reading	Controls (No Streptomycin Added)	1.0 μM Streptomycin at 0 Hours	2.0 μM Streptomycin at 0 Hours	2.0 μM Streptomycin at 6 Hours	2.0 μM Streptomycin at 12 Hours	2.0 μM Streptomycin at 24 Hours
	hours						
10.0	0	2	*2*	2	2	2	2
	6	15	10	8	*15*	14	14
	12	41	32 (22)*	11 (73)	32 (22)	*41*	43
	24	132	98 (26)	10 (92)	61 (54)	104 (21)	*135*
	36	218	136 (38)	9 (96)	55 (75)	155 (29)	200 (8)
	48	310	218 (30)	9 (97)	50 (84)	184 (40)	267 (14)
1.0	0	2	*2*	2	2	2	2
	6	13	7	6	*13*	13	13
	12	43	15 (65)	7 (84)	28 (35)	*44*	44
	24	133	20 (85)	9 (93)	41 (69)	104 (22)	*134*
	36	215	21 (91)	7 (97)	40 (77)	133 (38)	205 (5)
	48	309	22 (94)	5 (99)	36 (88)	144 (53)	259 (16)
0.1	0	1.5	*2*	2	2	2	2
	6	6	4	3	*6*	7	6
	12	24	8 (67)	4 (83)	11 (54)	*24*	25
	24	104	9 (91)	4 (96)	17 (84)	69 (34)	*106*
	36	180	9 (95)	4 (98)	20 (89)	75 (58)	155 (14)
	48	237	10 (96)	4 (98)	10 (96)	70 (70.5)	172 (27)

* The figures in parentheses represent the per cent inhibition of growth.

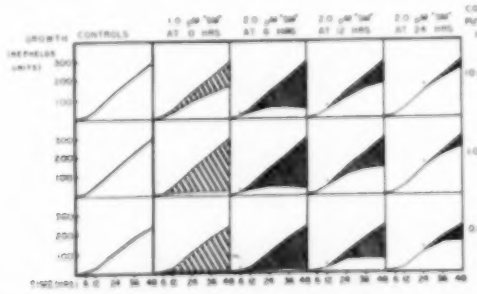

FIG. 2. Graphic representation of the effect of streptomycin (1.0 and 2.0 μM), when added at different times, on the growth of *Mycobacterium smegmatis* in a medium containing different concentrations of putrescine, with albumin (0.2 per cent). "SM" = streptomycin.

were used. Enough streptomycin was added to give the following concentrations: 1.0 and 2.0 μM of streptomycin at zero hours, and 2.0 μM of streptomycin at six, twelve, and twenty-four hours. In figure 2, 2.0 μM of streptomycin at zero hours is omitted, since the results in the presence of all three putrescine concentrations are quite similar.

It is seen that reduction of the amount of putrescine increases the inhibition of growth brought about by streptomycin, most strikingly seen with 1.0 μM of streptomycin at zero hours.

Further experiments showed that the omission of albumin does not affect the growth of this organism to any significant degree in putrescine- or ammonium sulfate-containing medium in either the absence or presence of streptomycin. The growth readings obtained in a medium in which ammonium ions were the sole source of nitrogen are set forth in table 3. Streptomycin was added at the same times and in the same concentrations as in the previous experiment with the

TABLE 3

THE EFFECT OF VARIOUS AMOUNTS OF STREPTOMYCIN WHEN ADDED AT DIFFERENT TIMES ON THE GROWTH OF MYCOBACTERIUM SMEGMATIS IN A MEDIUM CONTAINING AMMONIUM SULFATE AS SOLE NITROGEN SOURCE

Turbidimetric Readings in "Nephelos Units"

Streptomycin Concentration μM	Time of Reading	Controls (No Streptomycin Added)	Streptomycin Added at 0 Hours	Streptomycin Added at 6 Hours	Streptomycin Added at 12 Hours	Streptomycin Added at 24 Hours
	hours					
0.5	0	3	*3*	3	3	3
	6	15	13	*15*	15	15
	12	45	38 (16)*	41 (9)	*42*	43
	24	115	94 (18)	108 (6)	108 (6)	*115*
	36	172	155 (10)	160 (7)	165 (4)	170 (1)
	48	240	215 (11)	220 (8)	235 (2)	240 (0)
1.0	0	3	*3*	3	3	3
	6	15	11	*14*	14	14
	12	45	27 (40)	40 (11)	*43*	44
	24	115	80 (30)	100 (9)	104 (10)	*115*
	36	172	135 (21.5)	150 (13)	160 (7)	168 (2)
	48	240	187 (22)	200 (17)	208 (13)	215 (10)
2.0	0	3	*3*	3	3	3
	6	15	10	*14*	14	14
	12	45	17 (62)	30 (33)	*44*	45
	24	115	22 (81)	60 (48)	107 (7)	*115*
	36	172	37 (78)	93 (46)	150 (13)	165 (4)
	48	240	54 (77)	140 (42)	198 (17.5)	213 (11)
4.0	0	3	*3*	3	3	3
	6	15	9	*13*	14	15
	12	45	7 (84)	25 (44)	*43*	45
	24	115	7 (94)	29 (70)	95 (17)	*115*
	36	172	6 (96)	35 (80)	118 (31)	153 (11)
	48	240	6 (98)	34 (86)	138 (42)	188 (22)

* The figures in parentheses represent the per cent inhibition of growth.

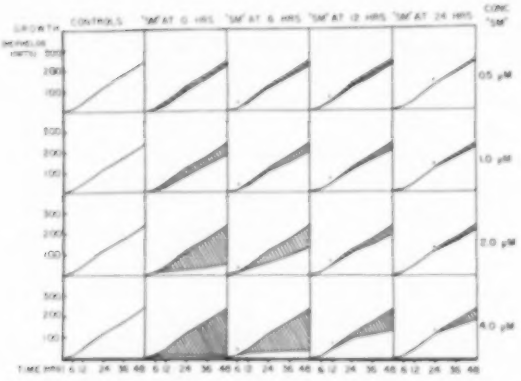

FIG. 3. Graphic representation of the effect of various amounts of streptomycin when added at different times on the growth of *Mycobacterium smegmatis* in a medium containing ammonium sulfate (40 μM NH_4^+) as the sole nitrogen source. "SM" = streptomycin.

TABLE 4

GROWTH OF MYCOBACTERIUM SMEGMATIS IN THE PRESENCE OR ABSENCE OF STREPTOMYCIN IN MEDIUM CONTAINING PUTRESCINE OR AMMONIUM SULFATE AS SOLE SOURCE OF NITROGEN

Turbidimetric Readings in "Nephelos Units"

Nitrogen Source	Time of Reading	Controls (No Streptomycin Added)	1.0 μM Streptomycin at 0 Hours	2.0 μM Streptomycin at 0 Hours
	hours			
Putrescine (10 mM)	0	3	2	2
	6	11	8	7
	12	36	31 (14)*	21 (42)
	24	107	81 (24)	21 (80)
	36	180	125 (31)	16 (91)
	48	249	165 (34)	31 (88)
Ammonium sulfate (5 mM) (10 mM ammonium ion)	0	3	3	3
	6	11	10	9
	12	29	23 (21)	18 (61)
	24	83	64 (23)	18 (78)
	36	153	105 (31)	25 (84)
	48	229	166 (28)	31 (87)

* The figures in parentheses represent the per cent inhibition of growth.

putrescine medium (table 1, figure 1). Figure 3 is a graphic representation of these data. The results show that the degree of growth inhibition at comparable streptomycin concentrations and times of addition is very similar in the two media. Two micromolar streptomycin when added at zero hours and at six hours was slightly more effective in the ammonium sulfate medium than in the putrescine medium.

A comparison is shown in table 4 between the growth in a medium with putres-

cine and ammonium sulfate, respectively, as the sole nitrogen source. The growth inhibition by streptomycin (1.0 and 2.0 μM at zero hours) is seen to be the same with these two nitrogen sources.

DISCUSSION

The growth-inhibiting effect of streptomycin was found to decrease regularly with successively later times of its addition. This might suggest that the inhibition observed is proportional to the amount of streptomycin per organism. When various portions of the experiment represented in table 1, however, are compared, it is found that similar microorganism/streptomycin ratios at different times of addition effect different degrees of growth inhibition. For example, the ratios are approximately the same for 1.0 μM of streptomycin added at six hours and 4.0 of μM streptomycin added at twelve hours. The per cent inhibitions for equal time intervals following addition of streptomycin, however, are 10 per cent and 23 per cent, respectively. This is also true in other instances recorded in table 1. If streptomycin at a concentration of 1 μM was added at twelve hours, the per cent inhibition after twelve hours was 8 per cent. When streptomycin at a concentration of 4 μM was added at twenty-four hours, the per cent inhibition after twelve hours was 31 per cent. In both instances, however, the organism/streptomycin ratio was approximately equal.

Therefore, it would seem that, on the basis of the microorganism/streptomycin ratio, the microorganisms appear to be more susceptible to streptomycin during the later part of the growth cycle. While it is apparent from figure 1 that the growth inhibition is more effective the earlier streptomycin is added, it is also true that much more streptomycin per cell is needed in the earlier stages of growth to effect a particular degree of inhibition.

When the concentration of putrescine in the medium is reduced, the growth-inhibiting effect of streptomycin increases.

The finding that streptomycin exerts approximately the same inhibitory effect in a medium containing ammonium sulfate or putrescine as nitrogen source permits the conclusion that inhibition of diamine oxidase does not explain the effect of streptomycin on the growth of this organism in general. One may draw from this the provisional conclusion that the growth-inhibiting action of streptomycin on *M. smegmatis* lies somewhere beyond the stage of uptake of ammonium ions by the organism, if indeed it has a primary action on nitrogen metabolism at all.

SUMMARY

Quantitative growth studies were carried out to determine the inhibitory effect of streptomycin added at various times during the growth cycle of *M. smegmatis*. The results show that a particular concentration of streptomycin is inhibitory to a greater degree the earlier it is introduced into the culture medium. Considering the ratio between the amount of bacteria and the number of molecules of streptomycin present, it seems that the bacteria during the early growth cycle are more resistant than later on.

Decreasing the concentration of available nitrogen in the medium increases

the effect of a given concentration of streptomycin. Organisms growing in a medium containing putrescine or ammonium sulfate, respectively, are inhibited to an approximately equal degree. It seems, therefore, unlikely that streptomycin susceptibility of *M. smegmatis* is based on inhibition of diamine oxidase by the drug.

SUMARIO

El Efecto de la Estreptomicina sobre el Crecimiento del Mycobacterium Smegmatis en Presencia de Putrescina o Sulfato de Amonio

Estos estudios cuantitativos del crecimiento tenían por fin determinar el efecto inhibidor de la estreptomicina agregada en varias ocasiones durante el ciclo del desarrollo del *Mycobacterium smegmatis*. Los resultados demuestran que una concentración dada de estreptomicina resulta más inhibidora mientras más pronto se introduzca en el medio de cultivo. Considerando la proporción entre la cantidad de bacterias y el número de moléculas de estreptomicina que hay presentes, parece que, durante el período temprano del ciclo de desarrollo, las bacterias son más resistentes que después.

La disminución de la concentración de nitrógeno disponible en el medio acrecienta el efecto de una concentración dada de estreptomicina. Los microbios que crecen en un medio que contenga putrescina o sulfato de amonio, respectivamente, se inhiben en forma aproximadamente igual. Parece, pues, improbable que la susceptibilidad del *M. smegmatis* a la estreptomicina se base en la inhibición de la oxidasa por la droga.

RESUME

L'effet de la streptomycine sur le Mycobacterium smegmatis en présence de putrescine ou de sulfate d'ammonium

Des études quantitatives sur la croissance ont été entreprises pour déterminer l'activité inhibitrice de la streptomycine introduite dans le milieu à différents moments durant le cycle de développement du *Mycobacterium smegmatis*. Les résultats obtenus démontrent qu'une concentration donnée de streptomycine a une action inhibitrice d'autant plus marquée qu'elle est introduite plus précocement dans le milieu de culture. Si on considère le rapport entre le nombre des bactéries et le nombre des molécules de streptomycine présentes, il semble que les bactéries sont plus résistantes durant la phase initiale de la croissance que plus tard.

La réduction de la concentration de l'azote contenu dans le milieu augmente l'effet d'une concentration donnée de streptomycine. Les germes cultivés cans un milieu renfermant, respectivement de la putrescine ou du sulfate d'ammonium sont inhibés à un degré sensiblement égal. Il semble donc peu probable que la sensibilité du *M. smegmatis* à la streptomycine soit fondée sur l'inhibition de l'oxydase diamine, par cet agent antimicrobien.

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Case Reports

THE FIRST CLINICAL TRIAL OF STREPTOMYCIN IN HUMAN TUBERCULOSIS[1, 2]

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The year 1954 marked the tenth anniversary of several special events in the chemotherapy of tuberculosis. Historical aspects of the discovery of streptomycin and the development of this substance as the first chemical agent found to be practical in the treatment of tuberculosis have been recorded for all time (1–3). To complete the story, the writers are presenting an account of the first clinical trial of the drug.

November 20, 1944, was the day on which streptomycin was first administered to a human being for the treatment of tuberculosis. The patient was a 21-year-old white girl who had progressive, far advanced pulmonary tuberculosis. When streptomycin was first administered to the patient, she was hospitalized at Mineral Springs Sanatorium, Cannon Falls, Minnesota. Later she was transferred to the Colonial (now Methodist) Hospital, Rochester, Minnesota, for continuation of treatment and study.

Those were early days in the chemotherapy of tuberculosis. In June, 1944, the writers terminated the first experiment, in which four guinea pigs infected with tuberculosis received streptomycin in small amounts for forty to fifty-four days. Both dosage and duration of treatment were limited by the supply of material. About the middle of September, 1944, a second experiment was terminated, in which nine guinea pigs received streptomycin in somewhat larger doses for forty-seven to sixty-one days. The third, or crucial, experiment to determine the therapeutic and toxic effects of streptomycin was still in progress when this patient received her first injection of the drug. However, the suppressive effect of streptomycin on progressive tuberculous infection had appeared so striking among the thirteen guinea pigs treated previously that everyone concerned, including the patient herself, was eager to try the new drug when her condition indicated the need for just such a suppressive effect.

When she was admitted to Mineral Springs Sanatorium in July, 1943, this patient (P.T.) had far advanced pulmonary tuberculosis involving the right upper lobe. During her first year of hospitalization she showed some improvement, but shortly afterward she began to have chills, fever, night sweats, and increased cough; a chest roentgenogram obtained in October, 1944, showed a pronounced increase in infiltration and cavitation in the right lung. This rather desperate situation appeared to call for desperate action, so thoracoplasty was performed on the right side by Dr. O. T. Clagett, of the Mayo Clinic.

The first stage of thoracoplasty was performed on November 1, 1944. Two weeks later

[1] When the work reported in this paper was begun, Drs. Pfuetze and Pyle were associated with the Mineral Springs Sanatorium, Cannon Falls, Minnesota; and Dr. Hinshaw was a member of the Section of Medicine, Mayo Clinic Rochester, Minnesota. Dr. Feldman was a member of the Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

[2] Presented at the Interim Scientific Session of the American Trudeau Society, New York New York, November 17, 1954.

roentgenographic study revealed a new and presumably an exudative lesion in the left lung. At this point it was decided that the patient's progressively unfavorable course made the use of streptomycin justifiable and desirable. Accordingly, some of the material then being used in the guinea pig experiment was appropriated for this first clinical trial.

As the therapeutic and toxic doses of streptomycin for human beings were entirely unknown, the drug was administered with extreme caution. At first the total daily dose was only 0.1 gm., and this amount was divided into eight doses given at three-hour intervals around the clock. From the first the drug was given by deep intramuscular injection. This treatment was often painful, being especially so because the patient was poorly nourished. The first preparations of streptomycin were relatively crude. The purity of different lots varied; but most of the material contained pyrogens and other substances such as histamine, so that, in addition to pain at the site of injection, the patient was likely to suffer from headache, flushing of the face, generalized muscular aching, pain in the joints, malaise, and sometimes chills and fever.

Between November 20, 1944, and April 7, 1945, the patient received five courses of streptomycin, each of which lasted ten to eighteen days. The treatment was interrupted, partly to give her relief from such side effects as those just listed, but mostly because at that time a steady supply of streptomycin was not available.

The first course of treatment was terminated on November 30, 1944. A month later the patient received another brief course which was terminated on January 12, 1945. During the first two courses, when the total daily dose was only 0.1 or 0.2 gm., it appears unlikely that any appreciable chemotherapeutic effect was achieved. In a roentgenogram obtained on January 13, 1945, the lesion in the patient's left lung appeared to be unchanged. At this time the total daily dose was increased, and during the last three courses, for a total of forty-four days, the patient received 0.5 to 1.2 gm. per day, a dosage which certainly was adequate in terms of body weight, which was only 75 pounds.[3] In a roentgenogram obtained on April 9, 1945, at the end of treatment, the lesion in the patient's left lung was lost to view.

While the patient was receiving chemotherapy, three more stages of thoracoplasty, including an anterior stage, were carried out. At the conclusion of her chemotherapy and surgical treatment, sputum still contained *Mycobacterium tuberculosis*. During the ensuing months, however, while a regimen of rest in bed was continued, the patient's sputum became consistently negative for acid-fast bacilli on microscopy and, finally (with occasional exceptions), cultures of sputum and gastric contents likewise became negative.

The patient was discharged from the sanatorium on July 13, 1947, with a diagnosis of apparently arrested pulmonary tuberculosis. Periodic examinations since that time have revealed no deterioration in her condition. In a thoracic roentgenogram taken February 26, 1954, the disease appeared to be inactive;[4] another film taken in August, 1954, was interpreted by the patient's home physician as showing no change from previous films.

The fact that this patient has remained well is particularly gratifying in view of the fact that she has led a very active life. After leaving the sanatorium, she married and is now the mother of three robust children who were born in 1950, 1952, and 1954, respectively. Inadequate as her chemotherapy was by present-day standards, sufficient evidence

[3] The dosage has been translated into the terms in use today. In the first experimental and clinical work, streptomycin was calculated in terms of "S" units, each of which was approximately equivalent to a microgram of pure streptomycin.

[4] The writers are indebted to Dr. Ezra V. Bridge, the present medical director of Mineral Springs Sanatorium, Cannon Falls, Minnesota, for preparing an abstract of the patient's medical record and for having her roentgenograms, including her most recent film, sent to us.

now exists to allow the conclusion that the streptomycin which this patient received in the first clinical trial of the drug was definitely beneficial and may even have been life-saving. It is a happy ending which has been duplicated tens of thousands of times in the streptomycin story.

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A RARE CASE OF SPONTANEOUS HEMOTHORAX[1]

LEO V. DiCARA

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INTRODUCTION

Spontaneous hemothorax is an unusual condition, being extremely rare in blood dyscrasias. Only four such cases have been reported to date. Two of these cases have been due to thrombocytopenic purpura. Pitt (1) reported one, and Freedman (2) reported the second. Accordingly, the following case is being reported.

E. K. S., a white male, aged 32, was admitted on February 2, 1953, in moderate shock to the Veterans Administration Hospital, Tucson, Arizona, for the first time. The past history reveals that, in 1939, a diagnosis of thrombocytopenic purpura was established. Since 1939, he had experienced severe gastric hemorrhages which had been attributed to a duodenal ulcer. In 1939, a splenectomy was performed to control gastrointestinal bleeding. At that time, the platelet count was 30,000 per cu. mm., with an erythrocyte count of 1,350,000 per cu. mm. Bleeding from the gastrointestinal tract continued intermittently, and a subtotal gastrectomy was performed in 1946. However, bleeding continued at intervals until 1950; then ceased abruptly. The patient had had no gastrointestinal hemorrhages since 1950. He developed severe allergic reactions to blood transfusions in January, 1950, and these had persisted to the date of hospital admission.

The family history was contributory in that the patient's mother died of cerebral hemorrhage at the age of fifty-seven. During life, she had a tendency to bruise and bleed easily. One sister died at the age of one year from a bleeding condition. The patient stated that, as a child, he bled easily from the nose. He was the father of six children, four boys and two girls. Two of the boys have bleeding tendencies.

Pulmonary tuberculosis was first diagnosed in September, 1951. The sputum was positive for *Mycobacterium tuberculosis*, and a lesion in the right upper lobe (figure 1) was demonstrable on roentgenograms. The patient was hospitalized at the Veterans Administration Hospital, Alexandria, Louisiana, and, beginning on October 25, 1951, he received 0.5 gm. of streptomycin and 12 gm. of para-aminosalicylic acid (PAS) daily for four months. He left that hospital against the advice of the staff in March, 1952, and came to Tucson, Arizona, in September, 1952.

On admission to the hospital, the chief complaint was pain in the right lower chest. The history revealed that the patient had become ill approximately forty hours prior to admission with increasing pain in the right lower anterior chest and dyspnea. He had been well for the past few months but, on the day of admission, he had a number of shocklike episodes with sweating and fainting. He was classified as being seriously ill at the time of admission. A chest film at this time was interpreted as showing pleurisy with fluid in the right base. No pneumothorax was seen on the roentgenograms (figure 2).

On the day after admission, aspiration of the pleural cavity revealed blood

[1] From the Veterans Administration Hospital, Tucson, Arizona.

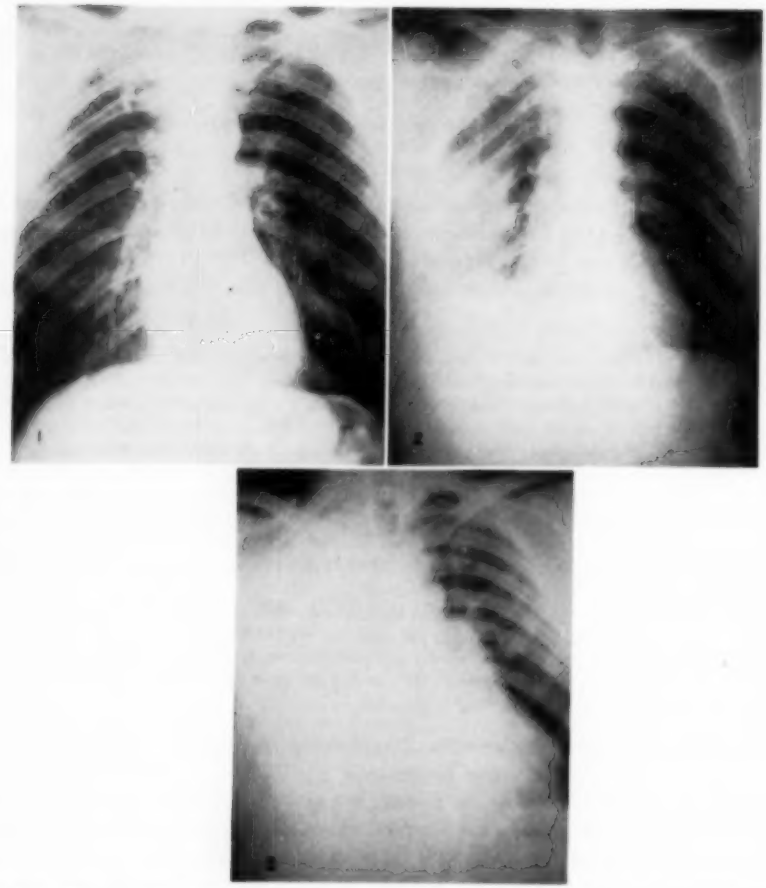


FIG. 1. Postero-anterior roentgenogram of chest, dated October 28, 1952.* Routine follow-up film in a patient with tuberculosis. Lesion is in the right upper lobe.

FIG. 2. Postero-anterior roentgenogram of chest, dated February 3, 1953,* showing large amount of fluid without any mediastinal shift or evidence of air.

FIG. 3. Postero-anterior roentgenogram of chest, dated February 10, 1953* showing marked amount of fluid and mediastinal shift. It was believed that adhesions in the right apex prevented completed mediastinal shift. (Note trachea is still in the midline.) It was at this point that intercostal catheter drainage was instituted.

in three different places in the right anterior lower chest. Approximately 15 cc. were removed and, following this, the patient went into shock. He was placed in an oxygen tent and given intravenous nikethamide (Coramine®). This was followed by a whole-blood transfusion of 500 cc. The transfusion was attended

* When the right intercostal spaces in all of the postero anterior views are compared, the progress toward normal may be followed.

with a moderate asthmatic reaction as it was being completed. The patient was semicomatose during most of the time in which the transfusion was being given. On the fifth hospital day, he complained of severe pain over the cardiac region. An electrocardiogram suggested pericarditis. A chest tap on the sixth hospital day yielded 200 cc. of nonclotting bloodlike fluid. A chest film (figure 3) on the seventh hospital day showed considerably more fluid with mediastinal shift to the left.

The diagnosis was bleeding in the right pleural cavity, secondary to thrombocytopenic purpura. Because of the history of severe allergic reaction to blood transfusions, it was thought best to delay intercostal drainage until the patient's general condition improved.

For the first six hospital days, the patient was in critical condition, and it was decided not to undertake any surgical procedures. On the sixth hospital day, a transfusion of 300 cc. of whole blood was given following administration of 30 mg. of diphenhydramine (Benadryl®) intramuscularly. He had a severe allergic reaction and required intravenous aminophylline. On the next day, 500 cc. of whole blood were given, followed by a more severe allergic reaction which required butanephrine and intravenous aminophylline in repeated dosage.

Special Rh factor and antibody studies suggested the possibility of an E factor as a cause of his reactions. The consulting pathologist suggested that the patient receive Rh-negative blood, which had been thoroughly warmed in an incubator, instead of Rh-positive blood.

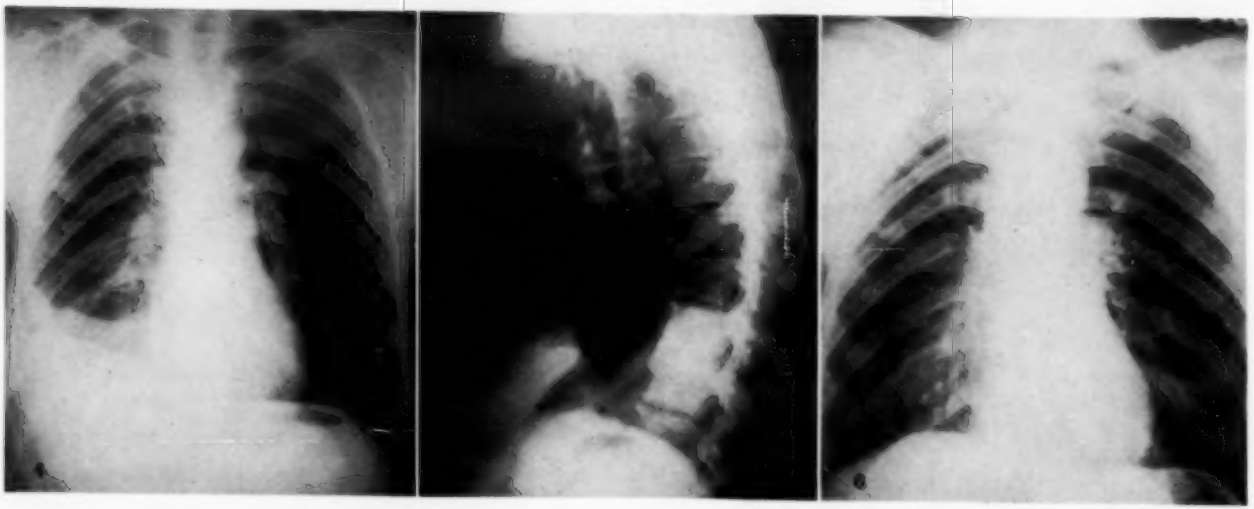
On the seventh hospital day, the patient was started on corticotropin, 40 mg. four times daily, along with streptomycin, 1 gm. daily.

Drainage of the right hemothorax was thought to be contraindicated at this time (February 10, 1953). A roentgenogram (figure 3) obtained on the same day showed considerable increase in mediastinal shift. The patient's condition was getting worse after only a slight improvement.

On the eighth hospital day, an intercostal catheter was inserted in the eighth interspace posteriorly, and 800 cc. of bloody fluid were immediately removed. A postero-anterior chest film following this procedure showed the mediastinum to be in the midline. The removal of 100 cc. of bloody fluid every hour through underwater seal drainage was continued, and the patient was constantly observed for any evidence of shock. A total of 2,400 cc. of blood was removed during the next forty hours.

On the fourteenth hospital day, 500 cc. of Rh-negative, specially warmed blood were started as a transfusion. After 250 cc. had been given, the patient developed chills and fever, but no asthmatic attack. Nevertheless, the transfusion was discontinued.

Because of this reaction, enzymatic therapy was delayed until the sixteenth hospital day. At this time, streptokinase-streptodornase (varidase), 100,000 units, were instilled in the pleural cavity and resulted in a drainage of 400 cc. of bloody fluid. Two subsequent instillations of enzymes, one on the next day and another on the twenty-first hospital day, were followed by the withdrawal of 2,000 and 1,600 cc. of bloody fluid. The intercostal tube was removed after nineteen days on March 2, 1953.



FIGS. 4 and 5. Posterolateral (figure 4) and right lateral (figure 5) roentgenograms of chest dated April 1, 1953,* showing marked improvement.

FIG. 6. Postero-anterior roentgenogram of chest, taken July 15, 1953,* showing almost complete resolution of the process. Actual film has slight blunting in the right costophrenic angle. A subsequent film, dated October 19, 1953, shows complete clearing.

* When the right intercostal spaces in all of the postero-anterior views are compared, the progress toward normal may be followed.

The patient was placed on deep-breathing exercises, and he made an uneventful recovery from this time on. A chest roentgenogram obtained on April 1, 1953 (figure 4) showed marked clearing of the densities in the right lower chest. A right lateral roentgenogram (figure 5) at this time failed to reveal any cavity in the right upper lobe. A follow-up roentgenogram of July 15, 1953 (figure 6) showed almost complete clearing of the process in the right base and an apparently stable lesion in the right upper lobe.

On admission to the hospital, the patient received penicillin, 600,000 units twice daily until March 2, 1953, and then 300,000 units twice daily until March 12, 1953. All sputum examinations were negative for tubercle bacilli. Two cultures of chest fluid for tubercle bacilli and for aerobic and anaerobic microorganisms were sterile. A bone-marrow biopsy on March 12, 1953, revealed no evidence of leukemia. The total amount of bloody fluid removed from the right chest was 6,600 cc., and the amount of blood given as transfusions was 1,550 cc.

In addition to the drugs already mentioned, the patient received vitamins C and K, B_{12}, iron, liver extract, Seconal®, Demerol®, codeine, and placebos. Corticotropin, which was recommended for transfusion reaction, also probably helped reduce capillary permeability, as has been reported by Ragan (3), Falcon and associates (4), and Meyers and associates (5). Amidopyrine was used in conjunction with the use of the enzymes, and the highest temperature observed was 102.6°F. The lowest leukocyte count was 58,700 cu. mm., with 93 per cent

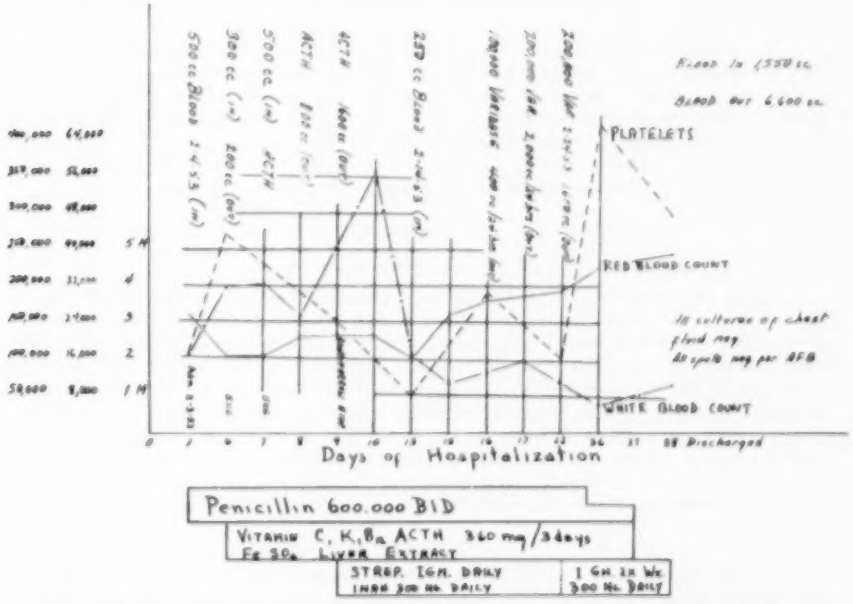


FIG. 7. Graphic representation of treatment of the case and some laboratory test results.

neutrophils. The lowest platelet count was 50,000 per cu. mm. on February 16, 1953 (figure 7).

On February 9, 1953, bleeding time was slightly increased, while coagulation time could not be obtained.

SUMMARY

A case of spontaneous hemothorax in a patient suffering from thrombocytopenic purpura and pulmonary tuberculosis is presented. Avoidance of any shocking procedures in the initial treatment of the case was believed to be very important.

Removal of blood can and must be timed to the patient's general condition, and accomplished as soon as feasible. It was believed that the use of streptokinase-streptodornase helped considerably in preventing a fibrothorax and the ultimate complete clearing of the right pleural space. The early use of breathing exercises following recovery is of paramount importance to obtain maximum lung function.

Etiology of spontaneous hemothorax is probably due to rupture of an adhesion or bullae necessarily involving vascular channels.

SUMARIO

Raro Caso de Hemotórax Espontáneo

Preséntase un caso de hemotórax espontáneo en un enfermo que padecía de púrpura trombocitopénica y tuberculosis pulmonar. Consideróse muy importante evitar todo procedimiento acompañado de choque en el tratamiento inicial del caso.

La extracción de sangre puede y debe ajustarse al estado general del enfermo, y llevarse a cabo cuanto antes sea posible. Al parecer, la varidasa ayudó considerablemente a impedir un fibrotórax y en el despejo total definitivo del espacio pleural derecho. El empleo temprano de ejercicios respiratorios después del restablecimiento reviste importancia primordial en la obtención de la función pulmonar máxima.

La etiología del hemotórax espontáneo radica probablemente en la rotura de alguna adherencia o de flictenas que afecta forzosamente los conductos vasculares.

RESUME

Un cas rare d'hémothorax spontané

Un cas d'hémothorax spontané chez un malade atteint de purpura thrombocytopénique et de tuberculose pulmonaire est présenté. L'auteur considère qu'il était très important d'éviter l'application de tout procédé violent dans le traitement initial de ce cas. L'extraction du sang peut et doit être faite aussitôt que possible en tenant d'abord compte de l'état général du malade. L'auteur pense que la varidase a considérablement facilité la prévention d'un fibrothorax, et permis éventuellement la libération complète de l'espace pleural droit. Les exercices respiratoires précoces pratiqués après la guérison sont d'une importance capitale pour permettre la récupération maxima de la fonction respiratoire.

L'étiologie du pneumothorax spontané est probablement la rupture d'adhérences ou de bulles interessant une région vascularisée.

Acknowledgments

The writer wishes to express his appreciation to Joseph Mineo and Lester Heitlinger of the Medical Illustration Laboratory, Veterans Administration Hospital, Tucson, Arizona, for the photographic reproductions of the graph and chest films.

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Notes

AN EVALUATION OF BLOOD BANK BLOOD AGAR MEDIUM FOR THE PRIMARY ISOLATION OF M. TUBERCULOSIS FROM SPUTUM AND GASTRIC CONTENTS

Recently Tarshis and Frisch[1, 2] recommended the incorporation of human blood into standard agar media to accelerate the growth of *M. tuberculosis*. The present study was undertaken to determine the value of this blood bank blood agar medium and of another later recommended by Tarshis for the primary isolation of *M. tuberculosis*[3].

In the first series in the present study, 2,659 routine specimens of sputum or gastric contents were inoculated on blood bank blood agar medium, American Trudeau Society (ATS) medium, and Löwenstein-Jensen medium. In a second series, 219 specimens of sputum from patients known to have recently been infectious were similarly inoculated on the later recommended blood agar medium,[3] ATS medium, and Löwenstein-Jensen medium. The behavior of all of the inocula was observed and recorded. Because of the practical importance of the data obtained, the pertinent details of the method of study and the results are herein reported.

Media: The blood agar medium for the first series was prepared according to the following formula: Bordet-Gengou agar base (Difco), 4.0 gm.; glycerol (analytical grade), 1.0 ml.; distilled water, 99.0 ml.; human blood bank blood containing ACD solution, 25.0 ml. The agar base was hydrated and dissolved by heating, then the glycerol was added, and the medium was sterilized in the autoclave for fifteen minutes at 15 pounds of pressure. After it had cooled to 50°C., blood bank blood, three or four weeks old, was added aseptically. The medium was dispensed into sterile screw-cap tubes, slanted, and incubated for forty-eight hours at 37° C. to test for sterility. The blood agar medium used for the second series consisted of: agar-agar, 1.5 gm.; glycerol (analytical grade), 1.0 ml.; pooled human blood bank blood containing ACD solution, 30.0 ml.; distilled water, 69.0 ml.; and penicillin, 50 γ per ml.

ATS medium and Löwenstein-Jensen medium were prepared in the laboratory according to specifications[4] and checked for sterility prior to use.

Specimens: In the first series, 2,659 routine specimens were inoculated from patients suspected of having tuberculosis or those who were under treatment for pulmonary tuberculosis. The patients on the tuberculosis wards were receiving chemotherapy consisting of streptomycin, para-aminosalicylic acid, iproniazid, and viomycin alone or in various combinations. From these patients, monthly specimens were obtained for culture. From patients who were on medical wards, sputum was collected in 24-hour specimens and, from those on tuberculosis wards, in 50-hour specimens, so as to contain three morning samples. In the second series, 219 specimens of sputum were inoculated from patients whose sputum had been known to contain tubercle bacilli in the recent past. Each specimen was collected in a sterile wide-mouthed bottle, to which an equal amount of 3 per cent sodium hydroxide was added. This was placed in a shaker for thirty minutes, followed by thirty minutes of incubation at 37.5°C. A 50 ml. portion was centrifuged at 3,000 r.p.m. for twenty minutes, the supernatant was decanted, and

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TABLE 1

RESULTS OF PRIMARY ISOLATION OF M. TUBERCULOSIS ON BLOOD BANK BLOOD AGAR MEDIUM, ATS MEDIUM, AND LÖWENSTEIN JENSEN MEDIUM

			Per Cent
Specimens positive on:			
At least one of the media		572	
All three media		289	48.93
Blood agar		332	58.80
ATS		462	80.80
Löwenstein Jensen		491	85.30
Growth of 332 specimens positive on blood agar:			
All three media		289	87.04
Blood agar and ATS		13	3.90
Blood agar and Löwenstein Jensen		22	6.60
Blood agar only		8	2.40
Growth appearing within three weeks on:			
Blood agar	of 332	81	24.39
ATS	of 462	113	24.45
Löwenstein Jensen	of 491	124	25.25
Contamination in 2,659 specimens on:			
All three media		36	
Blood agar		249	9.36
ATS		156	5.86
Löwenstein Jensen		110	4.13

the sediment was neutralized with normal hydrochloric acid containing 0.004 per cent phenol red indicator. The sediment was shaken, and 0.2 ml. was planted on each medium. The order in which the different media were inoculated was varied from day to day.

In the first series of 2,659 specimens inoculated, contamination of the tube occurred in all media in 36 instances; on the blood agar medium, in 249 instances; on the ATS medium, in 156; and on the Löwenstein-Jensen medium, in 110 instances. Of the total specimens, 572 were positive for *M. tuberculosis*. The positive cultures were obtained from 495 specimens of sputum and 77 specimens of gastric contents. These specimens were

TABLE 2

RESULTS OF PRIMARY ISOLATION OF M. TUBERCULOSIS ON BLOOD BANK BLOOD AGAR MEDIUM (SECOND), ATS MEDIUM, AND LÖWENSTEIN JENSEN MEDIUM

		Per Cent
Specimens positive on:		
At least one of the media	113	51.59
All three media	44	30.00
Blood agar (second)	70	31.50
ATS	82	37.44
Löwenstein Jensen	93	42.46
Growth appearing within two weeks on:		
Blood agar (second)	18	16.00
ATS	7	6.19
Löwenstein Jensen	9	7.96

derived from 15 patients on the medical wards and 138 patients from the tuberculosis wards. Only the patients on the tuberculosis wards were receiving antituberculous chemotherapy.

The colonies of *M. tuberculosis* appeared within the first three weeks in 81 tubes on the blood agar medium, in 113 on the ATS medium, and in 124 tubes on the Löwenstein-Jensen medium.

The cultures were positive on the different media as follows: 332 on blood agar medium, 462 on ATS medium, and 491 on Löwenstein-Jensen medium. Of the 332 specimens positive for *M. tuberculosis* on blood agar medium, 289 were positive on all three media, 13 were positive on blood agar medium and ATS medium, and 22 were positive on blood agar and Löwenstein-Jensen media, while 8 were positive on blood agar medium alone (table 1).

In the second series of 219 sputum specimens chosen from patients who were discharging tubercle bacilli in the sputum, the tubes were contaminated in 8 instances: 2 on the second blood agar medium, 6 on ATS medium, and none on Löwenstein-Jensen medium. Of the remaining 211 specimens, 113 were positive for *M. tuberculosis* on at least one of the media, 70 were positive on the second blood agar medium, 82 on ATS medium, 93 on Löwenstein-Jensen medium, and 44 on all three media (table 2). Of the 70 cultures positive on the blood medium, the colonies first appeared in 18 cultures between the fifth and fourteenth days of incubation. This occurred in 7 of the cultures on ATS medium and in 9 on the Löwenstein-Jensen medium.

From the results obtained, it is concluded that blood agar medium is not as suitable for the primary isolation of *M. tuberculosis* in patients receiving antituberculous chemotherapy as are the ATS medium and the Löwenstein-Jensen medium. Nevertheless, the results obtained in the second series suggest the merits of the second blood agar medium for the early isolation of *M. tuberculosis*.

In summary: An evaluation was made of blood agar medium, comparing it with the ATS medium and the Löwenstein-Jensen medium for the primary isolation of *M. tuberculosis*. The blood agar medium yielded fewer isolates of tubercle bacilli than the ATS medium and the Löwenstein-Jensen medium. However, the colonies of *M. tuberculosis* appeared earlier on the second blood agar medium than on any of the other media.

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POSSIBLE IMMEDIATE DELETERIOUS EFFECT OF ISONIAZID ON COURSE OF TUBERCULOUS MENINGITIS[1]

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

Subsequent to the introduction of isoniazid into the treatment of tuberculous meningitis, we have noted evidence of immediate deterioration of several patients. This, manifested by hyperpyrexia and exaggeration of the cerebrospinal fluid and neurologic changes, has occurred within twenty-four to ninety-six hours after 8 to 10 mg. per kg. per day of isoniazid has been started.

In table 1 are recorded pertinent observations on 17 infants and children with tuberculous meningitis. All were closely observed immediately before and following initiation of antimicrobial therapy. Nine received streptomycin, and 8 received streptomycin plus isoniazid. Of the former, only one showed a marked increase in fever within two days. Six of the 8 who received isoniazid became hyperpyretic within forty-eight hours. Increased abnormality of the cerebrospinal fluid occurred more often, earlier, and to greater degree in those who received isoniazid in addition to streptomycin.

TABLE 1

COMPARISON OF IMMEDIATE EFFECTS OF SPECIFIC THERAPY ON TUBERCULOUS MENINGITIS

Treatment	Streptomycin	Streptomycin and Isoniazid
Total number of patients	9	8
Hyperpyrexia	3	6
Time interval	1 in 2 days (+ 4°F)	5 in 2 days
	1 in 9 days	(+ 1° to 4°F.)
Cerebrospinal fluid worse	2	4
Time interval	1 in 2 days	1 in 1 day*
	1 in 6 days*	1 in 2 days*
		1 in 3 days*
		1 in 4 days
Clinically worse	3 (moderate)†	6 (3 marked)‡
	3 in 2 days	1 in 1 day
		3 in 36 hours
		2 in 2 days

* Marked rise in both cells and protein.

† Two with increased irritability and rigidity; 1 with deepening coma.

‡ Two with increased lethargy; 2 with convulsions; one with decerebrate rigidity; one with increased cyanosis.

These observations suggest that the profound changes may be manifestations of a Herxheimer-like reaction due to destruction, by two such potent sterilizing agents as isoniazid and streptomycin, of large numbers of multiplying tubercle bacilli with release of tuberculin.

Sincerely yours,
MARYETHEL MEYER
ARTHUR ROBINSON
GARDNER MIDDLEBROOK
ROBERT ALWAY

DEPARTMENTS OF PEDIATRICS
NATIONAL JEWISH HOSPITAL AND
UNIVERSITY OF COLORADO SCHOOL
OF MEDICINE
DENVER, COLORADO
November 15, 1954

[1] This project was supported, in part, by the United States Public Health Service, Grant No. E-123 (C_3).

A POSSIBLE ANTAGONISTIC EFFECT BETWEEN PNEUMOTHERAPY AND CHEMOTHERAPY

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

The letter written by Dr. Cutler is an excellent example of the same authority being used to prove opposing points of view, the article by Bjork (J. Thoracic Surg., 1953, *26*, 533) having been quoted in our original communication.

That the bronchial circulation with its anastomoses will keep the pulmonic tissues alive in a completely atelectatic lung is well known. The question at issue becomes: How adequate is this circulation for bringing therapeutic agents to the lung parenchyma in effective concentration? Obviously this question is more important when dealing with a relatively nondiffusible agent such as streptomycin than when an agent with a much smaller molecule such as isoniazid is being used. In his letter, Dr. Cutler mentions that " . . . the bronchial circulation contributes not more than 1 per cent to the total pulmonary circulation." Does this mean that an atelectatic area would get only 1 per cent of the amount of streptomycin, for example, that adjacent normally aerated tissue was receiving? With the passage of time and the opening of collaterals the proportion would undoubtedly become greater, but how much time would be required?

These are theoretical questions concerning which we do not pretend to have accurate information. Liebow and his associates (Am. J. Path., 1950, *26*, 177) found after ligation of the pulmonary artery in dogs that expansion of the bronchial collateral circulation was well advanced within twelve weeks, but twelve weeks may be a long time in the present-day treatment of tuberculosis.

In the early days of chemotherapy, when the close association between the open cavity and the development of resistant strains of *M. tuberculosis* was first noted, it was the practice at Maybury to attempt to induce collapse of the lung *before* giving streptomycin. Any such procedure we now believe to be erroneous in the present, more advanced day of antimicrobial therapy. We believe that the patient should have the benefit of an unimpeded pulmonary circulation, at least during the initial phase of chemotherapy.

What may be the ultimate synthesis of the best in pneumotherapy and chemotherapy, no one can predict at the moment. As stated in our first letter, the pendulum may have swung to the extreme in neglecting temporary collapse measures. At the same time we stand by our original contention that, particularly when streptomycin is used, " . . . pneumotherapy, if successful, may tend to inhibit specific therapy by way of the bloodstream."

Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium)
Northville, Michigan
January 14, 1955

Sincerely yours,
C. Eugene Woodruff
Wilbur J. Steininger
Edna M. Jones
Morris D. Kloppenstein
Edwin E. Mueller
W. Leonard Howard

Books

BIOCHEMICAL DETERMINANTS OF MICROBIAL DISEASES. By René J. Dubos. Pp. 152, Harvard University Press, Cambridge, Massachusetts, 1954, Price, $3.50.

In the great majority of studies of host-parasite relationships, there has been the tendency to consider the microbial agent, on the one hand, as it behaves in simple artificial media, and the reactions of the host, on the other, as the effects of its serum or plasma on the infectious agent. This leaves out of consideration the essential environment or ecology of the host-parasite interactions in the infected individual. The general *milieu interieur* which is reflected in the plasma is not the biochemical situation within the phagocyte in which most microbial agents find themselves *in vivo*; nor is it the condition of the locally infected tissues.

It is the special contribution of this illuminating and comprehensive monograph to elucidate some of the biochemical changes produced in the host at the site of infection which affect the proliferation of the microorganism, its essential structural antigenic components, the actions of its endotoxins and its phenotypic expressions, such as the production of its lethal exotoxins. It is these factors which, to a great extent, determine whether the degree of proliferation of the microbial agent will cause progressive disease or a mere silent infection. For, infection is widespread; disease is rare. This is true not only of tuberculosis and other bacterial infections but also of viral and even of microbial plant diseases. Nonimmunologic factors such as cortisone, sex hormones, radiation, and the presence of boron will determine whether latent infestations by *pseudotuberculosis murium*, Bitner's virus of mammary cancer, intestinal bacteria, or the crown gall microorganism, respectively, will remain inapparent symbionts or destroy the affected individual.

The virulence of human- and bovine-type tubercle bacilli for the rabbit and calf cannot be understood on the basis of the behavior of these bacteria on the current artificial media, but rather on the basis of the as yet unknown, subtle biochemical changes that take place at the site of infection in these species, changes which are deadly to the human bacillus, on the one hand, and are borne with impunity by the bovine bacillus, on the other.

The inflammatory area at the site of infection is the site of intensive glycolytic metabolism, of high carbon dioxide and low oxygen tension, where high concentrations of lactic acid accumulate and where the pH is markedly depressed. These local biochemical changes exercise a profound influence on the proliferation of the microorganism. When antigens and antibodies interact, there is a frequent activation of proteolytic and autolytic enzymes. The release of nucleic and fatty acids and certain basic amines at the site of tissue necrosis may influence the survival of the infectious agent and affect the bacteriostatic properties of antibiotics.

It is in this light that the author considers infection and disease, the fate of microorganisms *in vivo*, and the biochemical disturbances produced by infection. Special application is made to the action of endotoxin *in vivo*, the Shwartzman and anaphylactic reactions, the problem of tuberculosis, and the relationships between inflammation and immunity.

It is beyond the scope of this review to detail the many processes which Dr. Dubos integrates in such masterly fashion. What is clear is that it gives a powerful impetus to the analysis of the host-parasite relationships from the most fundamental biochemical

approach. It is this approach which promises a rich harvest of understanding, and, hence, eventual power in man's struggle against some of the hostile factors in his environment.

MAX B. LURIE
Philadelphia, Pennsylvania

THE SURGERY OF PULMONARY TUBERCULOSIS. By James H. Forsee. Pp. 208, with illustrations, Lea & Febiger, Philadelphia, 1954, Price, $6.50.

This monograph is a fine contribution because the concepts of the surgical treatment of pulmonary tuberculosis have radically changed in the short period since chemotherapeutic agents have been available. It will bring the reader up to date with the recent developments of the applicability of surgery in the treatment of pulmonary tuberculosis.

The monograph is divided into three parts. The first part discusses principles in the application of surgery to pulmonary tuberculosis. There is a section concerning the general aspects of the patient and his disease. This is followed by a review of the surgical aspects of pulmonary tuberculosis, with an excellent discussion by Major T. E. Puckett of the pathology of pulmonary tuberculosis in relation to surgical therapy.

Part two concerns the operative procedures. In a brief but comprehensive manner, the development and usual techniques of collapse therapy are described, including indication, complications, and results. Similarly, the techniques used in extirpative surgery such as lobectomy, pneumonectomy, segmental resection, and wedge resection are described. The factors determining the amount of lung to be removed are mentioned.

The longer third part of the monograph is devoted to the extensive experience of Colonel Forsee and his colleagues with the surgical therapy in pulmonary tuberculosis at Fitzsimons Army Hospital. The details of anesthesia, preoperative care, postoperative care, and the rarely mentioned but important physical rehabilitation of the postoperative thoracic patient are thoroughly described.

At greater length there are chapters relating the experiences of extrapleural thoracoplasty. These should be contrasted to the author's highly encouraging experiences with extirpative therapy, which since chemotherapy has found wider application in the treatment of pulmonary tuberculosis. Such topics as indication, distribution, and type of extirpative surgery, chemotherapeutic programs, length of hospitalization, and follow-up are thoroughly explored.

Throughout are interspersed illustrative case reports, photographs, tables, and graphs, which reinforce and clarify the text. The statistical material seems significant in view of the author's large experience.

There has been a great need for this book for all those who care for patients with pulmonary tuberculosis. The author is to be congratulated for his clear and informative expression on this difficult subject.

GEORGE E. WANTZ
New York, New York

INFECTIOUS MONONUCLEOSIS. Modern Medical Monographs, No. 5. By Sidney Liebowitz. Pp. 163, Grune & Stratton, Inc., New York, 1953, Price, $4.75.

This monograph, based primarily upon the author's observations in twenty-four successive sporadic cases, represents a fairly well-rounded summary of the current facts and concepts related to the syndrome of infectious mononucleosis. Major emphasis is placed upon the practical aspects of the clinical, hematologic, and serologic manifestations of the syndrome.

No attempt is made at a comprehensive review of the literature, although an extensive list of references is included.

It should be emphasized that the author's observations are from sporadic cases of infectious mononucleosis. The frequent occurrence of clinically inapparent infectious mononucleosis with hepatic involvement, hematologic, and serologic manifestations is not, therefore, given extensive consideration. Nevertheless, the fact that these observations are from sporadic cases of clinically apparent disease allows the author to emphasize many lesser known pathologic occurrences in this syndrome.

The opinions expressed by the author will be of great interest to those concerned with the many clinical facets of this syndrome. This monograph is well written and is enthusiastically recommended to those interested in infectious mononucleosis.

C. LeMaistre
Atlanta, Georgia

Atlas of Exfoliative Cytology. By George N. Papanicolaou. Harvard University Press, Cambridge, Massachusetts, 1954.

The *Atlas of Exfoliative Cytology* by Dr. George N. Papanicolaou is a masterful presentation of the field of exfoliative cytology by an expert.

This atlas is in loose-leaf form in order to allow for future addition and correction. In a new field such as exfoliative cytology, such a form is advantageous, since new concepts and techniques are appearing rapidly. However, librarians will view this format with a jaundiced eye, as loose-leaf books are notoriously difficult to keep intact.

The book is divided into three parts. First, there is a detailed description of technique. Each anatomic area requires a slightly different technique. The method of collection, fixation, and staining of smears from the female genital tract, of urine, sputum, gastric secretions, pleural and peritoneal fluid, breast secretion, and rectal and colonic washings is presented in a concise and clear fashion.

In the second part, Dr. Papanicolaou discusses in detail the criteria for malignancy in exfoliated cells. The criteria are presented in outline form and are under several headings: nuclear changes; cytoplasmic change; changes of the cell as a whole; criteria based on the interrelationship of cells; and, finally, indirect criteria. This section should be of great help to the student of cytology.

The third and largest section of the book is devoted to discussion, description, colored drawings, and photomicrographs of specific systems. These are the female genital, male genital, urinary, respiratory, and digestive systems. The cytology of pleural, peritoneal, and pericardial exudates is discussed, as are the specific cells seen in breast secretions. The colored drawings, done by Hashime Murayama, are good, and reproduced well. The colored photomicrographs are, in general, good, but an occasional group appears to lack detail. This may only be in contrast to the drawings in which detail is presented so well. Photomicrographs of groups of cells are difficult to do, since all cells are rarely in one plane.

The cells desquamated from the respiratory system are presented in groups. For example, twenty-three examples of the columnar cells seen in sputum and bronchial washings are presented on one page, so that the student may immediately see what characteristics they all have in common, and also how they may differ. This manner of presentation the author refers to as comparative cytology and it appears to be a useful method for students. Malignant cells seen in a case of alveolar-cell carcinoma, oat-cell carcinoma, and squamous carcinoma of the lung are shown—again in a single plate. Cells from metastatic lesions are also illustrated. The plates on the respiratory system cover also patho-

logic conditions of the lung, such as bronchiectasis, adenomatosis, bronchial cyst, and adenoma. The section on the respiratory system is complete and the illustrative cells are well chosen.

The later plates in this atlas are an even further extension of "comparative cytology." There are photomicrographs of multinucleated cells from various organs. The drawings of histiocytes from different sources are superb and, I venture to say, will become a classic in "comparative cytology."

This atlas is highly recommended to morphologists interested in the field of exfoliative cytology.

Ruth M. Graham
Boston, Massachusetts

BOOKS RECEIVED

An Historical Chronology of Tuberculosis. By Richard M. Burke. Pp. 125, Charles C Thomas, Springfield, Illinois, 1955, Price, $3.75.

Studies in Tuberculosis. By R. G. Ferguson. Pp. 124, University of Toronto Press, Toronto, Canada, 1955, Price, $3.50.

The Health Visitor and Tuberculosis. By Sheena H. Buchanan. Pp. 150, National Association for the Prevention of Tuberculosis, London, 1955, Price, $2.00.

Readings in Anthropology. By E. Adamson Hoebel, Jesse D. Jennings, and Elmer R. Smith. Pp. 417, McGraw-Hill Book Company, New York, 1955, Price, $5.00.

American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

SYMPOSIUM FOR GENERAL PRACTITIONERS ON TUBERCULOSIS AND OTHER CHRONIC PULMONARY DISEASES

The fourth annual Symposium for General Practitioners on Tuberculosis and Other Chronic Pulmonary Diseases will be held in Saranac Lake, New York, from July 11 to 15, 1955. It is approved for twenty-six hours of formal credit for members of American Academy of General Practice.

This five-day course is designed particularly for general practitioners and presented over a period short enough so that they may readily attend. Many of the sessions are informal panel discussions with ample opportunity for questions from the audience.

Sessions will be held in the various sanatoriums, hospitals, and laboratories in the Saranac Lake area. The faculty will consist of physicians, surgeons, and scientists from Saranac Lake as well as guest lecturers.

Many doctors attending previous sessions of this Symposium have brought their families with them to enjoy the many vacation facilities of the surrounding Adirondack Mountains. So that families may have the use of the family car, free bus transportation will be provided to the various meeting places for the doctors attending the course. Excellent housing accommodations are available in and around Saranac Lake.

The registration fee for the Symposium is $40.00. Further information and copies of the program can be obtained by writing Dr. Richard P. Bellaire, General Chairman, Symposium for General Practitioners, P. O. Box 2, Saranac Lake, New York.

ANNOUNCEMENT OF REGULAR CORPS EXAMINATIONS FOR MEDICAL OFFICERS UNITED STATES PUBLIC HEALTH SERVICE

A competitive examination for appointment of Medical Officers to the Regular Corps of the United States Public Health Service will be held in various places throughout the country *on June 7, 8, and 9, 1955.*

Appointments provide opportunities for career service in clinical medicine, research, and public health. They will be made in the ranks of Assistant and Senior Assistant, equivalent to Navy ranks of Lieutenant (j.g.) and Lieutenant, respectively.

Entrance pay for an Assistant Surgeon with dependents is $6,017 per annum; for Senior Assistant Surgeon with dependents, $6,918. Provisions are made for promotions at regular intervals.

Benefits include periodic pay increases, thirty days annual leave, sick leave, medical care, disability retirement pay, retirement pay which is three-fourths of annual basic pay at time of retirement, and other privileges.

Active duty as a Public Health Service officer fulfills the obligation of Selective Service.

Requirements for both ranks are U. S. citizenship, age of at least twenty-one years, and graduation from a recognized school of medicine. For the rank of Assistant Surgeon, at least seven years of collegiate and professional training and appropriate experience are needed, and, for Senior Assistant Surgeon, at least ten years of collegiate and professional training and appropriate experience are needed.

Entrance examinations will include an oral interview, physical examination, and comprehensive objective examinations in the professional field.

Application forms may be obtained by writing to the Chief, Division of Personnel, Public Health Service, Department of Health, Education, and Welfare, Washington 25, D. C. Completed application forms must be received in the Division of Personnel *no later than May 6, 1955.*

ANNUAL MEETING OF THE AMERICAN COLLEGE OF CHEST PHYSICIANS

The Twenty-First Annual Meeting of the American College of Chest Physicians will be held at the Ambassador Hotel, Atlantic City, New Jersey, June 1 through 5, 1955. The scientific program will include approximately 200 speakers representing specialists in all aspects of diseases of the heart and lungs. In addition to formal presentations, the program comprises a number of symposiums, round-table luncheon discussions, diagnostic-treatment conference, and motion pictures. More than the usual amount of time has been allotted for open discussion.

A new feature this year will be the Fireside Conferences, to be presented on Friday evening, June 3. At this session more than thirty experts will be present to lead the discussions on as many subjects of current interest in the specialty of diseases of the chest.

Fellowship examinations will be held on June 2, and on Saturday evening, June 4, more than 100 physicians will receive their Fellowship certificates at the annual Convocation, which will precede the Presidents' Banquet.

All interested physicians are cordially invited to attend the Twenty-First Annual Meeting of the College; there is no registration fee. Copies of the program may be obtained by writing to the Executive Offices, American College of Chest Physicians, 112 East Chestnut Street, Chicago 11, Illinois.

ASIAN-PACIFIC TUBERCULOSIS CONFERENCE

The Asian-Pacific Tuberculosis Conference will be held in Sydney, August 15 to 21, 1955, the week preceding the Australasian Medical Congress.

As well as Interstate visitors and representatives of South-East Asian countries, other eminent delegates will be attending from North America, the United Kingdom, and Europe, including Dr. Johannes Holm, Chief of the Tuberculosis Section, Division of Communicable Diseases, of the World Health Organization. The program includes discussions on BCG vaccination and tuberculosis control and treatment.

A limited amount of accommodation will be available for visitors in Wesley College, at the University of Sydney. Further information may be obtained from: Dr. Bruce Geddes, Conference Secretary, The Royal North Shore Hospital of Sydney, Crows Nest, New South Wales, Australia.

TENTH ANNUAL SCHERING AWARD COMPETITION OPENS FOR NATION'S MEDICAL STUDENTS

The tenth annual Schering Award competition for medical students has opened, it was announced by Dr. Robert W. Burlew, chairman of the award committee. Its aim is to encourage medical writing and exploration of current research literature.

Titles of three subjects on which American and Canadian students are invited to submit papers this year are: Current Concepts in the Management of Osteoporosis; Prevention

and Treatment of Blood Transfusion Reactions; and Recent Trends in the Clinical Use of Adrenocortical Steroids.

Both a $500 first prize and a second one of $250 will be awarded for each of the three subjects. Outstanding authorities in each field will select the winners. Special recognition in the form of a professional gift will be given each student submitting a meritorious paper.

Deadline for entry forms specifying the choice of title is July 1. Manuscripts should be mailed not later than October 1. Students may compete individually or cooperatively in teams.

The Schering Award Committee brings outstanding papers to the attention of appropriate professional journals. Several papers by award winners of recent years have already been published in journals.

Dr. Burlew said that publication of these papers demonstrates the high level of research and medical writing attained by many participants in this traditional competition.

Information and instructions for the award competition are available from the Schering Award Committee, 60 Orange Street, Bloomfield, N. J.

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Foreword by René J. Dubos, Ph.D. and Walsh McDermott, M.D.

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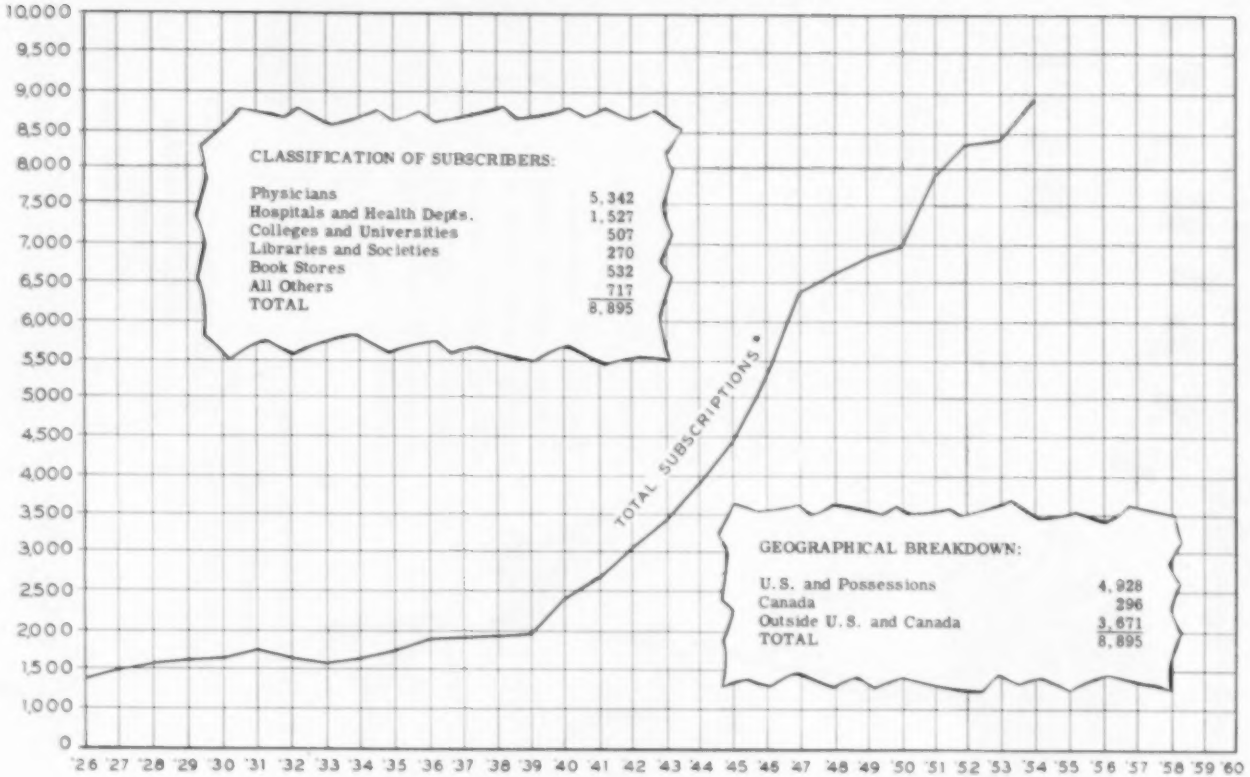

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	Mild	*Moderate*	*Total*
Streptomycin	0	0	0
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*Heck, W.E.; Lynch, W.J., and Graves, H.L.: Acta oto-laryng. 41:416, 1953.

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1. *Rudin, L. N., and Jensen, J. R., C. M. Digest, XX: 7, 1953*
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by E. M. MEDLAR, M.D.

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1. Hyman, S., and Rosenblum, S.: Illinois M. J. 104:27, 1953.
2. Papper, S. E., and Sutton, F. W.: Antibiot. Med. 2:188, 1954.

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